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THE UNIVERSITY OF ALBERTA

REPRODUCTIVE AND DIGESTIBILITY STUDIES IN THE EQUINE

BY

© EDWARD IAN SUTTON

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

IN

ANIMAL NUTRITION
DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA
FALL, 1976

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled " Reproductive and Digestibility Studies with the Equine" submitted by Edward Ian Sutton, in partial fulfillment of the requirements for the degree of Master of Science.

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Date May 26, 1976

ABSTRACT

Two experiments were conducted dealing with equine nutrition. The first experiment, conducted at Olds College, involving eight pregnant and four open mares had the primary objective of evaluating the relative adequacy of NAS-NRC (1973) Nutrient Requirements for Horses as they applied to mares receiving minimal shelter under central Alberta weather conditions. In the second experiment conducted at the Edmonton Research Station, seven geldings were used to compare the total fecal collection versus the insoluble ash technique for determination of apparent digestibilities.

In the reproductive experiment, mares were randomly placed in two groups. The first group received a diet designed to meet NAS-NRC (1973) requirements. The second group received the same diet but at levels of 15% less, by weight. The dietary regime was maintained from early gestation until 30 days post partum for both groups.

No significant ($P \quad 0.05$) differences were found for mare weights or for rate of gain, birth and 30 day weights for foals. Only three of the twelve measured blood constituents showed any significant difference. Blood urea nitrogen concentration was greater ($P < 0.05$) in group 2 than group 1 foals. Plasma cholesterol concentrations were greater ($P < 0.05$) in group 1 than group 2 foals. Plasma total protein concentration was greater ($P < 0.05$) in group 1 than group 2 mares. Analyses of colostrum and milk samples showed no significant differences for the constituents measured except for milk protein samples which were greater ($P < 0.05$) in group 2 than group 1 mares.

It was concluded in the reproductive study, for the phases of reproduction measured, that the low level diet 85% of NAS-NRC (1973) did not differ from the high level diet in terms of maintenance for pregnant mares.

The second experiment, involving a rate of passage of digesta trial and three digestibility trials used a diet identical to that which was used in the reproductive experiment for digestibility Trial 1. Trials 2 and 3 as well as the rate of passage trial used a diet consisting of 1/3 dehydrated alfalfa pellets and 2/3 crushed oats.

Digestibility studies with geldings revealed no significant differences regarding method of determination. Standard deviations tended to be greater in determinations derived from total collections than from the insoluble ash technique. No significant differences were encountered for random-grab versus periodic sampling with the insoluble ash technique.

The digestibility trial provided evidence suggesting that the random-grab insoluble ash technique

was not significantly different and, in fact, was superior to the total collection method, in terms of consistent estimates for the determination of apparent digestibilities.

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INTRODUCTION

In Canada, as in the rest of North America, man's use of the horse has changed over the past three decades (Ensminger, 1969). The horse, once valued as a work animal on the farm has become a source of recreation almost to the exclusion of its past functions. With this change, the light horse has replaced the heavier, draft breeds.

As a result of these fundamental changes in the horse population and scientific advances regarding nutritional requirements of other domestic species, much of the pre-1965 research has had to be re-evaluated and updated. Not only has past research needed further examination, but the research that was available regarding nutrient requirements of horses was scant.

Recently, there has been a tremendous increase in the area of establishing specific nutrient requirement guidelines for horses. The studies over the past ten years have been forced to deal with the most basic physiological and digestive functions of horses. Consequently, information pertaining to the nutritional requirements of horses under extraordinary conditions have largely been derived from extrapolated data of other species and subjective evaluation. Reliable data for nutrient requirements of horses have been slow to accumulate, largely because it is difficult to maintain the large numbers of animals necessary for establishment of statistically significant results. It was in response to the critical need for additional information that these reproductive and digestibility studies with horses were begun.

Reproduction is necessary to the maintenance and development of animal populations. The effects of, and requirements for nutrientsare of critical importance to the efficiency and success of this phase of the animal's life cycle. Unfortunately, very little research dealing directly with nutrient requirements of the reproductive phase in horses is available. Consequently, it was felt that studies of pregnant mares with particular emphasis on the evaluation of NAS-NRC (1973) nutrient requirement guidelines, would provide valuable quantitative information for the further refinement of requirement guidelines for horses.

A second extremely important facet of research in the field of nutrition is the determination of digestibility of feeds for a given animal. A great deal more information is needed in this regard for horses. Determinations of these digestibilities is a costly and time consuming affair. As a result, few digestibility studies with horses have been completed. It is for the purpose of reducing the cost and time involved for determinations of digestibility that new methods of determination have been developed. It was decided that digestibility studies comparing two of these methods of determination would be of value to the overall study of nutrition in horses.

LITERATURE REVIEW

Reproductive Study

There is scant research work available on nutrient requirement of mares that can be applied to current conditions. Early work referred to by Olsson (1949), Olsson and Ruudvere (1955) indicate that mares during gestation need no additional nutrients above maintenance until the seventh month of gestation. All agree that the period of lactation represents the interval of highest nutritional requirements. However, at that time, there was considerable disagreement as to how the requirements should be estimated. In spite of this obvious need for further clarification, reviews (Robinson and Slade, 1974; Hintz, 1969) indicate that there has been little recent research conducted in this field.

Determination of Nutritional Requirements in the Pregnant and Lactating Mare

Historically, there has been very little research done relating directly to horses. The establishment of specific nutrient guidelines for pregnant and lactating mares is even less well defined. With very few exceptions, the recommendations (NAS-NRC, 1973) are a compromise between early observations and extrapolations of more recent work with non-pregnant horses and pregnant and lactating cattle (Robinson and Slade, 1974). Virtually the only exception to this, for pregnant mares, is work by Ott (1970) referred to by Robinson and Slade (1974) which indicated that dietary protein levels have a direct influence on the maintenance of body weight by brood mares and on birth weights of foals. Estimates of nutrient requirements (NAS-NRC,1973) for energy and protein have been adjusted partially on the basis of more recent work with digestibilities.

Parameters Used to Measure Performance in Horses

Horses more than any other farm animal have been the focal point of much confusion over what constitutes superiority in the animal. Unlike cattle or other more conventional farm animals, the horse in North America is not a meat producing animal. In fact, the most popular use for horses today is recreational, which may or may not entail heavy work. The parameters used to judge the performance of a horse, in modern application, are subject to subtle and often confusing interpretation. As a result, diets which, scientifically, meet the particular requirements of a horse may not be adequate for the show ring where many intangibles are considered (Wagoner, 1973). The major purpose of publishing nutrient requirement guidelines is that they will be used by horse owners. Therefore, the credibility of the guidelines rests solely on what parameters are initially used to measure relative performance. I do not wish to pursue this line of thought any further at this time, except to point out that the parameters we have chosen to measure in this trial were intended to relate only to basic performance and were not intended to apply necessarily to the more subtle traits of the show horse.

(a) Weights

One of the first symptoms of an inadequate diet in any animal is a loss in weight (Guyton, 1969; Maynard and Loosli, 1969). However, not all nutritional deficiencies are immediately reflected by weight loss (Maynard and Loosli, 1969). Therefore, while the recording of weights in mares and foals is basic to the evaluation of a ration it cannot be assumed to be the only necessary measurement.

(b) Blood Measurements:

Working in 1969 with some sixty light horses and ten ponies, Medway (1969) obtained results from blood analyses, as shown in Table 1 and 2. Somewhat different results from electrophoresis were obtained by Wolff et al (1969), Table 3. The latter found, by comparison with previous literature, that their results fell within the range of previously established normal levels. Similarly, the results of Kirkham et al (1969) obtained blood analysis results from 247 mares, some of which were pregnant. The values obtained for serum calcium and phosphorus agreed

Table 1. Results of Plasma and Serum Chemical Studies on 60 Light Horses of Mixed Breeds

	BUN[1]	Gluc[2]	TP[3]	Total[4] Bili	Direct[5] Bili	SGOT[6]	LDH[7]	ALK[8] Phos	Ca[9]
	mg%	mg%	g%	mg%	mg%	Sigma Units	Sigma Units	Sigma Units	mg%
Maximum	39.4	120	9.9	4.0	0.30	840	1500	7.5	14.4
Minimum	14.9	55	5.2	0.4	0.00	142	350	2.0	9.5
Mean	25.08	77.2	7.28	1.83	0.09	252.3	686.2	4.08	11.73

[1] Blood Urea Nitrogen

[2] Plasma Glucose

[3] Plasma total protein.

[4] Total Bilirubin - as measured from plasma

[5] Direct Bilirubin - as measured from serum

[6] Serum Glutamic - Oxaloacetic transaminase

[7] Lactic Dehydrogenase

[8] Alkaline Phosphotase

[9] Plasma Calcium

(Medway 1969)

Table 2. Results of Paper Electrophoresis on Serum from 32 Horses.

	TP	Alb[1]	Alpha$_1$[2]	Alpha$_2$[3]	Beta[4] and Gamma[5]	A/G[6]
Maximum	8.00	4.30	1.36	1.86	2.95	1.43
Minimum	5.80	2.29	0.65	0.65	1.16	0.46
Mean	7.07	3.06	0.96	1.22	1.85	0.79

[1] Serum Albumin

[2] Alpha - 1 - globulins

[3] Alpha - 2 - globulins

[4] Beta globulins

[5] Gamma globulins

[6] Alpha: Gamma globulin ratio

(Medway 1969)

Table 3. Blood Serum Values of Horses of Different Ages.

Age	T Pg/100 ml	Alb	Alpha$_1$	Alpha$_2$	Beta$_1$	Beta$_2$	Gamma	A/G
8 yrs.	7.7±0.8	2.22±0.2	0.72±0.13	1.09±0.21	1.05±0.21	0.91±.2	1.75.34	.41±0.07
4½ - 8 yrs	7.5±0.7							
2½ - 4½ yrs.	7.2±1.0							
2½ yrs.	7.2±1.0							

(Wolff et al 1969)

with those of Medway (1969). Unfortunately, Kirkham and co-workers did not separate the results obtained for pregnant mares from the other mares. As a result, no information which could be applied specifically to pregnant mares, was available. There does not appear to be any information published to date on blood constituents relating specifically to pregnant mares.

In an effort to measure early response of mature horses to changes in diet, Fonnesbeck and Symons (1969) measured some blood parameters in six geldings being fed successively six different types of roughage. They found that plasma urea nitrogen concentrations were greater when diets of highest protein content were fed. Plasma protein concentration was not found to change in response to dietary protein levels. This observation has been supported by Reitnour and Treece (1971). Plasma glucose concentrations were found to increase in response to diets high in soluble carbohydrates. The rapid response of plasma glucose concentrations to soluble carbohydrates is considered attributable to the fact that the horse, in contrast to the ruminant, is able to digest and absorb soluble carbohydrates without subjecting them to fermentation (Alexander, 1954). Similar results with regard to blood glucose levels were obtained by Crawford et al (1970). However, Hintz et al (1971 b) found that blood glucose levels were not influenced by the forage-grain ratio. Furthermore blood glucose concentrations have been shown to be unaffected by exercise, gestation or lactation (Evans, 1971). However, blood glucose concentrations in the horse have been shown to rapidly decrease under fasting conditions (Alexander, 1954) and (Evans, 1971).

Recent work with infusion trials show that glucose turnover was 2.8 times higher than reported for lactating mares (Anwer et al, 1975). Furthermore, it was found that 90% of the lactose in milk is derived from plasma glucose and that milk composition accounted for 40% of the glucose turnover in lactating mares. Consequently, it is not surprising that relatively wide variations were noted for plasma glucose concentrations. Presumably, these variations are concomitant with milk production of individuals.

The measurement of blood plasma constituents has, in the past, been of value in evaluating sources of feed and the subsequent overall metabolic pathways involved. In addition, these same constituents have been used for the detection of disease stress, especially from responses in serum calcium, serum creatine and lipoprotein values (Gainer et al, 1966; and Wolff et al, 1969).

(c) Milk and Colostrum

In one of the few recent experiments dealing with the composition of mares' milk, Ullrey et al (1966)

Table 4. Constituents of Mares' Milk*

Stage of Lactation	Total Solids gm/100gm	Crude Protein NX6.25	Lipids gm/100gm	Lactose gm/100gm	Gross Energy kcal/100gm	Calcium mcg/gm	Phosphorus mcg/gm
Partum	25.2	19.1	0.7	4.6	135	847	389
12 h	11.5	3.8	2.4	4.8	64	782	399
24 h	11.4	3.3	2.5	5.2	62	973	442
48 h	12.0	3.3	2.5	5.8	62	1110	457
5 days	11.5	3.1	2.1	5.9	59	1199	444
8 days	11.5	3.1	2.0	5.9	59	1278	441
3 weeks	11.3	2.7	2.0	6.1	56	1261	391
5 weeks	11.2	2.7	2.3	5.7	59	1110	325
4 months	10.0	2.0	1.3	6.5	49	614	216

*Taken from Ullrey (1966)

found that values for gross energy, specific gravity, total solids, crude protein, ash, magnesium, sodium and potassium decreased abruptly following parturition to 12 hour post partum and then tended to decline slowly throughout lactation (Table 4). Lactose concentrations tended to increase throughout lactation. Levels of nutrients found by Ullrey et al (1966) are in general agreement with those presented by Altman (1961). Iron, zinc and copper values obtained from mares' milk were found to be similar to those reported for other species (Ullrey et al, 1974). However, when iron and copper concentrations are compared with NAS-NRC (1973) estimates of dietary requirements, the values obtained from these milk samples appear to be low. Ullrey et al (1974) suggested that deficiencies of this nature are rarely found. Either foals exhibit lower than estimated requirements or natal reserves combined with milk intake supply sufficient iron and copper to prevent deficiencies.

Contrary to what was once suspected, 'foal heat' scours associated with the mares' first estrus after parturition has not been found to be related to any changes in composition of mares' milk (Johnston et al, 1970).

In order that we might maintain our perspective with regard to the nutritive value of mares' milk, the information presented in Table 5 shows the estimated

Table 5. Comparisons of Milk Constituents for Mares and Cows

	Mare	Cow
Dry Matter (%)	9.4	12.8
Crude Protein (%)	2.0-2.4	3.5
Carbohydrate (lactose)(%)	5.9	4.9
Lipids (%)	1.1	3.7
Ash (%)	0.4	0.7
Digestible Energy (kcal/kg)	540	720

(Morrison, 1946)

and(Ensminger, 1969)

nutrient composition of mares' milk as it compares with estimates of cows milk (Morrison, 1946; and Ensminger, 1969).

Nutritional research has been late in coming to the horse family. As late as this last decade the differences between horses and cattle have been minimized (Olsson, 1949 and Olsson and Ruudvere, 1955). In come cases, the Equidae has been completely ignored from the discussion of animal nutrition (Maynard and Loosli, 1969). Furthermore, until recent years, the majority of research work in horse nutrition has been of the qualitative type. However, current research is attempting to be more precise in its attempt to define the nutrient requirements of horses.

Anatomy of the Digestive Tract

The Equidae, like other grazing animals, have evolved special adaptations for their survival. One of these areas of special adaptation has been in the digestive tract, allowing it to utilize diets high in cellulose. Like other herbivores, the horse is dependent on a microbial population within the digestive tract, capable of cellulytic digestion in order to utilize diets high in fibre. In the ruminant, the specialized area for bacterial activity is a multi-chambered stomach located anterior to the small intestine (Alexander, 1952). Horses, on the other hand, possess an enlarged cecum and colon situated posterior to the small intestine. The essential differences associated with the digestive tracts of the horse compared with ruminants are that in the former, (1) there is no rumination, (2) there is no eructation, (3) the rate of passage of digesta is more rapid, (4) food material is exposed to enzymatic digestion prior to fermentation, (5) microbial cell bodies are not exposed to enzymatic digestion in the small intestine, (Robinson and Slade, 1974).

It is interesting to note that estimates of the concentration of bacteria in cecal fluid of horses can vary from 10^{10} to 10^{11}/g ingesta (McCreery et al, 1971). These microbial populations are similar to those occurring in the rumen of steers on a mixed diet. However, the proportions and types of bacteria are somewhat different and there are fewer caecal protozoa present in horses (Kern et al, 1973).

Digestion

By studying parotid saliva secretions in two ponies, Alexander (1966) found that saliva is secreted only during the mastication of food. In these two ponies the average 24 hour flow of parotid saliva was 5.6 litres.

Alexander was able to show that concentrations of sodium, potassium, chloride and calcium in parotid saliva, increased with the rate of saliva flow.

The rate of passage of food material has been shown (Alexander and Benzie, 1951) to be more rapid in the adult horse than in the foal. This suggests that gastric digestion plays a less important role in the adult than in the foal.

In a comparison of the gastro-intestinal tracts of ponies and steers, it was found that fermentation does occur in the stomach of the horse. Proteolytic activity was found to be greater in the ileum of ponies than in steers. Protozoa were detected in the rumen of steers and cecum of ponies but not in the cecum of steers. These studies indicate that in ponies the colon may have more total proteolytic and as many cellulolytic bacteria as the cecum (Kern, et al, 1973). This bacterial population in the cecum and colon of ponies, has been shown to be essential for the effective utilization of plant fibre (Knapka, 1967). Conversion to volatile fatty acids (VFA) and ammonia is a possible fate of the bacterial amino acids associated with cecal and colon microbial activity (Wysocki and Baker, 1972).

The addition of oats to hay diets of horses, resulted in both an increase in the total number of bacteria and in specific species of bacteria, (Kern et al

1973). Robinson and Slade (1974), point to other work which suggests that the cecum and colon are major sites of absorption where sodium, chloride, bicarbonate, VFA and amino acids are removed. Furthermore, Fonnesbeck, (1968 b) found that the ventral colon acts as the major site of water absorption.

Nutrients

(a) Rate of Passage

Owing to the differences in the digestive tracts of horses compared with ruminants, horses show measurable differences in the rate of passage of digesta compared with cattle and sheep. VanderNoot et al (1967) found that the cumulative recovery of chromic oxide was maximal from 36 to 48 hours and generally complete by 72 hours. This agrees with work cited by Olsson and Ruudvere (1955). Using coloured styrofoam particles, Hintz and Loy (1966) found that horses beig fed a pelleted ration excreted an average of 61% of all particles ingested by 27 hours. However, horses on a non-pelleted ration excreted only 29% of all particles within 27 hours. Using chromic oxide with six ponies, Haenlein et al (1966 b) estimated rate of passage of digesta to take approximately 48 hours. Pulse et al (1973), found that retention of both chromic oxide and polyethylene indicators was increased following cecal fistulation. From these and other studies it is evident that apparent rate of passage of digesta is dependent on both the type of feed (Haenlein et al 1966 a, and Hintz and Loy, 1966) and the type of indicator used. Haenlein et al,(1966 a) suggest that while the physical form of the feed used will influence the rate of passage, the actual diet composition does not. It appears that the overall rate of passage of digesta in equine species is intermediate between small non-ruminants, such as pigs, and ruminants.

Work by Alexander (1966) and Alexander and Benzie (1951) suggest that in spite of a more rapid rate of passage of digesta on the part of horses compared with ruminants, the reduction in digestibility coefficients is not comparable. This would imply that cellulolytic activity is more efficient in the equine cecum than in the ruminant rumen. This tends to support earlier work by Balch and Johnson (1950) which showed slower rates of disappearance of suspended cotton threads in the rumen than in the equine large intestine.

It has been reported that cattle will digest protein more efficiently than horses. (Olsson and Ruudvere, 1955; Olsson, 1949; Kennedy, 1972; and Knox, 1971). I believe there is reason to dispute the universal application of this suggestion. The results of these workers showed that for specific horses receiving specific rations, cattle tended to utilize protein more efficiently. There is, on the other hand, abundant evidence to suggest that horses should not be compared with cattle on the basis of typical cattle feeds.

Work in the U.S.S.R. by Andreyev (1971) suggests that ranges not able to support cattle are often used for horse meat production. Fonnesbeck (1967) found that there were large differences between individual horses in their ability to utilize proteins. In my opinion the discrepancy lies in the fact that the equine evolved on a highly varied diet. Consequently, when forced to consume a ration with two or three protein sources, the horse does not show the efficiency it may have shown under natural conditions where rates of passage of digesta have been found to be slower (Haenlein, 1966 b).

(b) Sites of Digestion

Several studies have been made with equine species to determine the sites of digestion and absorption. Unfortunately much of this data has been calculated using chromic oxide as an indicator. In light of the failure of this technizue with ruminants (Drennan et al, 1970) and horses (Knapka et al, 1967) much of the following information must be treated with a certain amount of skepticism.

Hintz et al (1971 a) sectioned the digestive tracts of eleven ponies immediately after they were killed. By calculating chromic oxide nutrient ratios for each section they were able to estimate digestion coefficients of dry matter (DM), crude protein (CP), available carbohydrate (ACHO) and neutral detergent fibre (NDF). They found that the major site of ACHO and CP digestion was prececal; whereas the major sites of NDF digestion were the colon and cecum regardless of the hay-grain ratio being fed. From these results they suggested that the relative importance of digestion in the lower gut increased with increased levels of roughage.

Working on the comparative digestibility of components of forages by geldings and steers, VanderNoot and Galbraith (1970) found that as the ratio of crude fibre increased in the diet, the digestibility of protein by horses relative to steers decreased. However, they were not able to show a similar relationship with cellulose digestion. They suggested that protein digestion is predominantly prececal. Reitnour et al (1970) reported studies in agreement with both Hintz and VanderNoot. Finally, Kern (1974) found that proteolytic activity was greater in the small intestine than in the large intestine of the horse.

With regard to vitamin and mineral absorption, Schryver et al (1972); Hintz et al (1973 a,b) and Argenzio and Lowe (1974) have suggested that the major sites of phosphorus absorption are in the small and large dorsal colon. Calcium, along with magnesium, appears to be absorbed predominantly in the small intestine, (Hintz and Schryver, 1972 b, 1972 c). Little if any work has been reported on the sites of absorption for other minerals.

(c) Digestion Coefficients

As has been pointed out earlier, the number of digestion trials with horses and the variety of feeds evaluated are limited. However, there has been a certain amount of information published. In a review by Olsson and Ruudvere (1955) they refer to earlier work on over 1000 digestibility trials, predominantly with heavy horses, to suggest that the relationship between the digestibility coefficient for organic matter (y) is related to the fibre content (x) by the following equation:

For horses $y = 97.0 - 1.26x$

For cattle $y = 86.0 - 0.66x$

The fact that horses apparently are less efficient in digesting crude fibre than cattle, has been further substantiated by VanderNoot and Galbraith (1970), Slade and Hintz (1969) and Fonnesbeck (1968 a). In a review article by Robinson and Slade (1974), they referred to work by Hintz in 1969 that suggests that "tables of digestibility obtained with ruminants could be used directly for formulating horse rations when the feed contained less than 15% crude fibre". Above 15% crude fibre, rabbit data would underestimate and ruminant data would overestimate the value of the diet for horses.

Other workers have noted similar changes in digestibility coefficients. Applegate and Hershberger (1969) studying in vitro and in vivo techniques found that the crude fibre content was the single best indicator by step-wise regression of digestibilities. Later studies by VanderNoot and Trout (1971) came to similar conclusions.

Fonnesbeck (1968 b) found that water intake of horses, like cattle was closely related to dry matter intake. However, diets having a higher cell wall constituent content also caused an increase in water intake.

(d) Energy:

Since the horse has historically been considered a work animal, there has been an abundance of early research on the utilization of energy sources by equines. These studies have been referred to by Olsson and Ruudvere (1955), Morrison (1956), Nelson (1961) and Olsson (1949). Olsson (1949) points out that energy requirements are usually expressed in terms of maintenance and production requirements in units of kilocalorie (kcal) of digestible energy (DE).

The quantitative estimate of energy required for maintenance is derived from the expression: $E_m = aW^{0.75}$. In this model E_m is in kilojoules (kJ) of DE per day, a is a constant dependent on the stage of maturity of the animal, and W is the live weight in kilograms, (Pearce, 1975). A variety of values for a, has been calculated for mature horses. For example, a was calculated to be 727 by Albert (1959), 477 by Knox et al (1971), 594 by Wooden et al (1970) and 652 by Stillions and Nelson (1972). The reason for the sizable range in estimates may be due to the use of different types of horses, different measurement techniques and to differences in the amount of prior activity of the horses being studied. Also, it should be pointed out that the value of a, when calculated from a predetermined E_m, acts as a pool for all variations between individuals and therefore may be expected to vary considerably. The current recommendation of NAS-NRC (1973) allows for "normal activity of a non-working horse" and uses 648 kJ DE/$W^{0.75}$. Thus the maintenance requirement of a horse weighing 450 kg would be 63,280 kJ DE/day (Pearce, 1975).

Estimates of energy required to do work have been done by Hintz et al (1971 c) and are shown in Table 6. From this it can be seen that compared with walking, "strenuous effort" can require almost 80 times as much DE/hour. While these estimates have been accepted by the NAS-NRC (1973), it should be noted that factors such as weight of rider, environmental temperature, temperament of the horse and previous conditioning will influence the energy requirements for work.

Robinson and Slade (1974) refer to work by (Blaxter 1967; and Carlson et al 1964) that suggest

"Although glucose is more efficiently used than VFA's as a source of energy in ruminants, which of these substances is preferentially oxidized in equines and the capacity for utilization at the tissue level has not been clearly established." They continue to point out that both free fatty acids (FFA) and glucose can be utilized as an energy source. This is at least partially substantiated by Goodman et al (1973). Using four mature geldings, Goodman et al compared the response to exercise by both conditioned and unconditioned horses. They found that both fat and carbohydrates serve as sources of energy for the unconditioned horse. Furthermore, they found that 1/2 hour of trotting represented a measurable stress in the unconditioned animal. This stress was found to elevate plasma FFA and depress muscle glycogen. The conditioned horse was not greatly affected by the stress of trotting. Goodman et al (1973) suggested that this resistance to stress was due to more operative homeostatic mechanisms in the conditioned horse.

Evans (1971) found that plasma glucose concentrations were unaffected by exercise, gestation or lactation when compared with a control group. However, a 72 hour fast reduced the plasma concentrations. Exercise or lactation were found to cause a two-fold increase while fasting caused a two-fold decrease in irreversible loss of plasma glucose. This would suggest that the horse has the ability, during fasting, to alter its metabolism so that substrates other than glucose may be used as a source of energy. Robinson and Slade, (1974) refer to other studies to suggest that plasma glucose concentrations in the horse are intermediate between those of ruminants and monogastric animals. Diets high in soluble carbohydrates have been shown to elevate blood glucose levels in the horse. (Crawford et al, 1970). The implication of these results is the suggestion that horses are not solely dependent on gluconeogenesis for glucose, as are ruminants. Therefore it may be expected that horses are more efficient users of soluble carbohydrates for energy than are ruminants. Support for these findings was presented by Hintz, et al (1971 b) when they found that most soluble carbohydrates were digested in the small intestine to produce glucose and other simple sugars which are absorbed in the small intestine. Microbial fermentation of fibrous material, together with a small proportion of soluble carbohydrates, occurs in the cecum and colon with the production of VFA's (Stillions et al, 1970; Hintz et al 1971 b); these are absorbed through the walls of the cecum and colon.

Working with seven horses, Hintz et al (1971 a) estimated energy requirements of working horses (Table 6). Where this energy came from appeared to depend on the animal's metabolism rather than diet, as no changes in blood glucose levels were found in response to diet,

Table 6. Digestible Energy Requirements above Maintenance for Various Activities

Activity	Requirement	
	kJ/hr/$W^{0.75}$	Kcal/hr/kg mass
Walking	2.1	0.5
Slow trotting, some cantering	21.3	5.1
Fast trotting, cantering, some jumping	52.3	12.5
Cantering, galloping, jumping	100.3	24.0
Polo practice and games	163.0	39.0

(Hintz et al, 1971; Pearce 1975)

although glucose entry rates were found to be higher in grain rations compared with hay rations (Hintz et al, 1971 b). Subsequent work by Argenzio and Hintz (1972) showed that glucose oxidation rates will increase directly with entry. It would appear from this information that glucose is a preferred substrate for oxidation. However, under fasting conditions these workers found that the oxidative requirement for glucose was comparable to that of ruminants. The work by Argenzio and Hintz (1972) led them to suggest that equines fed high grain diets will adopt a pattern of metabolism similar to monogastric animals and those fed high fibre diets a pattern similar to ruminants. In addition to an ability to alter its pattern of energy metabolism, the horse appears to have a peculiar mechanism for glucose homeostasis, distinctly different from ruminants, (Argenzio and Hintz, 1970). Mehring and Tyznik (1970) suggest that the removal rate of exogenous glucose from the blood of ponies is slower than non-ruminants but faster than adult ruminants. A further finding by Argenzio and Hintz (1970) was that fasted animals have a lowered glucose tolerance. This has been substantiated by Ginochio (1973) in his work with glucose turnover in Shetland ponies.

Since the initial demonstration that large quantities of VFA are present in the blood of horses, little work has been done to establish the relative significance of VFA, (Robinson and Slade, 1974).

(e) Protein

Information on nitrogen metabolism in the horse appears to be rather confused, centering around the discussion of whether or not equines have the ability to utilize non-protein nitrogen (NPN) sources. In this regard, I will attempt to present the literature in approximate chronological order, in order that we may examine the matter in the light of progressively more recent research.

There is still some divergence of opinion as to precisely where the major site of protein digestion occurs. Robinson and Slade (1974) refer to the question as being one of small intestine vs. large intestine.

Fonnesbeck and Symons (1969) later supported by Johnson and Hart (1974), found that the level and source of dietary nitrogen will influence such parameters as plasma urea and plasma protein levels.

Pearce (1975) suggested that primary breakdown of soluble proteins occurs by the secretion of proteolytic enzymes in the stomach with most of the digestion and absorption of amino acids taking place in the small intestine. Support for this comes from such workers as Reitnour et al (1970) and Hintz et al, (1971 b). However, prior to 1970, Reitnour et al (1969) using chromic oxide indicators found that the majority of protein digestion took place in the cecum and colon. Slade and Robinson, (1970) went one step further and suggested from their data that the ability of the horse to digest protein was potentially equivalent to that of ruminants. This implied that there was extensive microbial activity in the equine gastro-intestinal tract, presumably in the cecum and colon areas. It was later found that large intestine fermentation of previously undigested proteins results in the formation of ammonia, amino acids and protein (Hecker 1971, and Slade et al, 1971). Based on the earlier findings of Slade and Robinson (1970), Slade et al (1971) supplemented urea to protein deficient diets and found an improvement in nitrogen balance and digestibility. They were also able to show improvements of a similar nature with complete diets.

Fonnesbeck and Symons (1969) suggested that the improvement in diets supplemented with urea was in fact due to microbial synthesis of proteins from the urea. Based on these earlier findings, Slade et al (1971) proposed that a nitrogen cycle similar to that of ruminants exists in horses. This hypothesis was further supported by suggestions from Wysocki and Baker (1972) that a possible fate of bacterial amino acids could be conversion to VFA and ammonia. Indirect criticism of this hypothesis was brought forward by Know (1971) when he found that digestible crude protein (CP) requirements for his two horses on test exceeded NAS-NRC (1966) guidelines by 95%. Knox failed to find support from Nelson and Tyznik (1971) when they suggested from their data that the horse was able to utilize urea as a NPN source and further, there appeared to be evidence of ammonia absorption from the cecum.

The extent to which horses can utilize sources of NPN is still not clearly established. Alexander (1966) found that concentration of urea in parotid saliva of horses was similar to that of sheep. However, total amounts of saliva secreted by the horse were found to be significantly less than sheep. Pearce (1975) refers to Houpt and Houpt (1970) as suggesting that salivary urea is absorbed in the small intestine and utilized in the liver to synthesize non-essential amino acids. It has been found that dietary urea does not reach the cecum via the digestive tract but that recycled urea in the blood is used to promote microbial activity in the cecum (Slade et al, 1971; and Nelson and Tyznik, 1971). Cecally infused urea has been found to increase concentrations of amino acids in the cecal fluid and in plasma, (Johnson, 1972 a,b). Reitnour and Treece (1971) did not obtain any benefit from the addition of dietary urea, but Hintz and Schryver (1972 a) showed that a small proportion of dietary urea was utilized. However, the efficiency of this utilization was less than for nitrogen derived from dietary protein.

It has been found that under certain circumstances, horses can tolerate large quantities of dietary urea, (Ratcliff et al 1963) but when Hintz et al (1970) fed 450 g urea to 8 ponies after 24 hours of fasting, 7 of them died.

There is considerable confusion regarding the need for dietary essential amino acids in mature horses. However, for an immature horse weighing 225 kg a requirement of 0.75% lysine has been found, (Breuer and Golden (1971) suggest that a growing horse weighing 215 kg requires about 29 g lysine/day, which is approximately 0.6% of its ration). The implication of this, is that the immature horse is substantially more dependent on prececal nitrogen than nitrogen derived from microbial fermentation in the cecum.

More recent work by Kennedy and Hershberger (1974) regarding microbial fermentation, suggests that while micro-organisms of the lower tract improve the biological value of low quality protein, protein thus formed may be poorly absorbed from the cecal and colon regions. Reitnour and Salisbury (1972) found with four cecally fistulated ponies that nitrogen from cecally infused protein was less efficiently utilized than the nitrogen from protein administered orally.

Finally, Kern et al (1971, 1973) present data to show that the microbial populations necessary for microbial synthesis are present in the intestinal tract of the equine. These observations indicate the potential for utilization of NPN sources in the horse.

The overall function of the digestive system in the horse is obviously not well defined. However, the most prevalent theory is that while the cecum and colon are largely responsible for the absorption of nitrogen, they are not solely responsible for the digestion of proteins. This leaves us with two alternatives. The first is in support of Slade et al, (1970), where it is suggested that the stomach and small intestine degrade nitrogenous portions of feeds, particularly soluble proteins, subsequently facilitating microbial fermentation. Therefore, by-passing the stomach and small intestine would be expected to reduce the effectiveness of microbial fermentation. The second alternative is that a significant amount of nitrogen absorption takes place in the stomach and small intestine. Therefore, prevention of this absorption would naturally reduce the total amount of protein digestion that will take place in the animal. In general, it may be stated that horses are affected by the protein quality of a diet but are not totally dependent on dietary protein source. Thus the dependence on dietary protein is intermediate between ruminants and monogastrics.

(f) Minerals

Horses are assumed to require the same minerals as other animals. Estimates of requirements for horses are largely derived from experiments with cattle. Some recent work defining mineral requirements for horses are: Stowe, (1967, 1968); Schryver et al, (1970 a, b); Stowe, (1971); Hintz et al, (1971 d); Schryver et al, (1972); Hintz and Schryver (1972 a, b) Hintz et al, (1973 a, b); Argenzio et al, (1974) Schryver, et al, (1974).

Dietary requirements depend upon the live weight of the animal, the amount of work, stage of maturity, the loss in sweat and the availability of each mineral in the diet.

(i) Calcium and Phosphorus

The levels of calcium required for maintenance is recommended to be 45 mg/kg live weight/day and for phosphorus 30 mg/kg live weight/day, (NAS-NRC 1973). Teeter et al, (1967) suggested requirements equivalent to 167-222 mg/kg live weight/day for calcium and phosphorus in expectation of low availability in the types of feeds normally fed to horses. Schryver et al (1970 a) reported calcium availability to be approximately 40 - 50%, while Whitlock et al (1970) obtained availabilities up to 70% from grass hay pellets, falling to 45% with the addition of limestone. Hintz and Schryver (1972 b) fed a wide range of feeds providing minerals at levels close to requirements and found mean calcium availability of 72% and phosphorus availability of 43%. Overall availability of phosphorus over a wide variety of levels of intake were found to be about 45%, (Schryver et al, 1971 b). Hintz, et al (1973 b) found that in many cereal grains and their by-products, a proportion of the phosphorus occurs as phytin which, despite the probable action of a phytase produced by cecal bacteria, is less available than in organic phosphorus.

The absorption of calcium occurs predominantly in the small intestine, (Schryver et al, 1970 b). Significant levels of phosphorus have been found to be absorbed in the large as well as the small intestine (Schryver et al, 1972). Furthermore, these workers suggest that the efficiency of utilization depends on the Ca:P ratio, the chemical form of the elements, the vitamin D status of the animal and interactions with other minerals. The optimum ratio for calcium and phosphorus, is suggested by Schryver and Hintz (1972) to be 1.4:1 to 2:1.

Calcium absorption has been found to be reduced by high intakes of phosphorus (Schryver et al, 1971 a), while high levels of calcium did not appear to influence phosphorus absorption. High levels of calcium intake (over 400 mg/kg live weight /day) have been shown to significantly reduce magnesium absorption (Whitlock et al, 1970).

Loss of calcium and phosphorus due to light exercise in horses has been estimated (Schryver et al, 1972) to be from 250 to 1000 mg calcium and 100 to 300 mg phosphorus per hour.

(ii) Magnesium

The requirements of horses for magnesium have recently been estimated to be 1.3 g/100 kg body weight/day (Hintz and Schryver, 1973). Requirements of magnesium for growth are estimated at 12 to 18 mg/kg body weight gain/day (Schryver et al, 1974).

Magnesium has been found to be almost exclusively absorbed from the small intestine with little or no absorption from the large intestine (Hintz and Schryver, 1972). These same workers also found mean availability of dietary magnesium to be approximately 50%, a level of availability which decreased in the presence of high levels of phosphorus in the diet. Whitlock et al, (1970) found that high levels of calcium tended to decrease the availability of magnesium.

(iii) Sodium Chloride

Pearce (1975) refers to work by Popov (1946) suggesting that horses may lose 50 - 60 g sodium chloride in sweat and 35 g in urine daily. Presumably, losses of sodium chloride may be greater under conditions of excessive heat or heavy work.

(iv) Selenium

Horses are susceptible to selenium toxicity (Robinson and Slade, 1974), called 'alkali disease'. The levels in the feed at which toxicity occurs has not been quantitatively established. However, in areas where selenium is as high as 5 - 40 ppm in natural feeds, characteristics of alkali disease may develop.

A condition known as 'tying up' has been found to be alleviated by addition of selenium to the drinking water, (Stewart, 1960). The requirements for selenium (Stowe, 1967 a) are estimated to be 240 ug/100 kg body weight/day.

(v) Zinc

Requirements for zinc are not known for horses (NAS-NRC, 1973). However, zinc deficiencies have been reported in growing horses being fed a diet of 4 ppm of zinc, while a diet of 40 ppm appeared to prevent deficiency (Harrington et al 1973, cited by Robinson and Slade,1974).

(vi) Iodine

Requirements for iodine are estimated to be 0.10 mg/day (NAS-NRC, 1973). Excessive levels of iodine have been noted in pregnant mares and causes goiter in the mares and foals (Baker and Lindsey, 1968).

(vii) Copper

Low levels of copper have been tentatively linked with hemorrhaging especially in older pregnant mares (Stowe, 1968 a). Precise requirements have not yet been estimated for horses.

(viii)Lead

Robinson and Slade (1974) refer to recent work by Schmitt et al (1971) as suggesting that growing horses may be particularly susceptible to lead toxicity. However, no specific toxicity or requirement levels have been estimated for horses to date.

(ix) Fluorine

Toxic effects of excess fluorine can occur. As a result it has been recommended that dietary levels of fluorine be held below 50 ppm/day (NAS-NRC 1973).

(x) Other Minerals

Requirements by horses for the remaining trace elements have not been determined, nor have there been any studies relating specifically to horses for these elements (Robinson and Slade,1974).

(g) Vitamins

Very little research has been conducted to establish precise vitamin requirements of pregnant mares. Here again, most of the recommended requirements have been estimated from work with non-pregnant animals.

Work by Garton et al, (1964) has shown that seasonal fluctuations in liver, fat and blood concentrations of carotene and vitamin A occur in horses. Early researchers have noted symptoms of vitamin A deficiency in horses (Olsson and Ruudvere, 1955). Results reported by Stowe (1968) show that 9.0 - 11.0 IU vitamin A per kg body weight per day for maintenance and an additional 50 IU vitamin A per kg body weight for pregnancy and lactation would prevent deficiency symptoms (Robinson and Slade, 1974).

Stowe (1967) suggests that vitamin E in combination with vitamin A appears to enhance reproductive performance in mares. Robinson and Slade, (1974) refer to other work that suggests that vitamin E has the potential to alleviate 'dry coat' in horses in the tropics. Furthermore, an average of 233 mg of oral alpha-tocopherol/ kg body wt./day was required to maintain erythrocyte stability.

Vitamin D has been shown to have a positive relationship to calcium and phosphorus absorption in horses. (Hintz et al, 1973 a).

It has been suggested (Stillions et al, 1971 a) that vitamin C is not a dietary essential for horses. There have been numerous reports. (Robinson and Slade, 1974) that horses are at least partially dependent on dietary sources of the B vitamins. Recent work by Stillions et al, (1971 b) found that at no time over a period of 11 months of a vitamin B_{12}- deficient diet were vitamin B_{12} deficiency symptoms noted. However, they were able to show that labelled cobalt is absorbed in the large intestine of the horse. Other B vitamins such as thiamin, riboflavin, pantothenic acid and niacin are assumed to be synthesized in the cecum of the horse; although there appears to be some conflicting evidence, (Robinson and Slade, 1974).

Methods Used for Determination of Digestibilities

Over the past decade there have been numerous attempts to utilize methods other than total collection to estimate the digestibility of feeds. Some of these have met with limited success while others have given misleading results.

Since it is impractical to carry out feeding trials with all of the different feeds and combinations which can be used to make a ration and since these trials measure only the final effect of the diet; other measures of nutritive value which give us more definite information as to why a particular result was obtained, are used. One of the most valuable measurements, is an estimate of digestibility of a feed, since undigested nutrients cannot be absorbed into the body.

A digestion trial involves a record of the nutrients consumed and of amounts voided in the feces. From these measurements, a quantitative measure of the nutrients absorbed by the body can be made. Various methods are employed for the collection of the feces. In the case of monogastric animals an indigestible 'marker' may be used. However, one of the critical assumptions that is made when using a marker or indicator, is that it is physiologically inert and will not diffuse through the gastro-intestinal tract; that is, that the marker will travel through the tract at the same rate as the nutrients.

This assumption for some markers has not been valid as we will discuss in more detail later. Some markers that are used in digestion trials are carmine, ferric oxide, chromic oxide and soot (Maynard and Loosli, 1969).

While digestion trials, involving total collection of feces over an extended period of time, are valuable, they are also very costly in terms of time and labor. Very few thorough digestion trials have been done with horses with the exception of Olsson in 1949 (Olsson and Ruudvere, 1955). Recent research, which involved the determination of digestibilities, has, for the most part, used chromic oxide as an indicator. (Hintz et al, 1971 b and Hintz et al, 1971 c). Chromic oxide was, in the past, considered to be a fairly accurate indicator. However, considerable doubt was cast on this assumption when workers such as Haenlein et al, (1966 a, and 1966 b) found that fecal chromic oxide samples taken at random from horses, provided estimates of fecal excretion rates which did not agree with those from total collection procedures.

Working in 1970 with cattle and sheep, Drennan et al (1970) supplied a variety of markers to estimate digestibility. They found that chromic oxide gave estimates of starch digestion ranging from 56 to 92%. In view of the unreliable results obtained using chromic oxide, they suggested that estimates based on lignin content of the feed would give more reliable results.

Knapka et al (1967) found that recovery of chromic oxide was only 81.5% as compared with 96.6% and 103.2% of labelled Ce^{144} and polyethelene respectively. Pulse et al (1973) found that cecal fistulation of horses will cause both chromic oxide and polyethelene to be retained longer. This apparently is due to an overall decrease in the rate of passage of digesta.

Most recently there has been the development of an alternative method of estimating fecal nutrients, which until now has only been used for the determination of apparent digestibility in pigs and poultry (McCarthy et al 1974) and (Vogtmann et al 1975). This method involves the use of HCL insoluble ash as an index material. The fact that the use of this material appeared to give more reliable results than chromic oxide when compared with total collection, has encouraged other workers to apply this method to other animal species such as poultry (Vogtmann et al 1975) and cattle (Van Keulen et al 1976).

Once the collection of feces has been made, an analysis of the nutrients remaining in the feces as well as those in the feed must be completed before estimates of digestibility can be made. The most common method used to determine the nutritive constituents of feeds is by proximate analysis (Maynard and Loosli, 1969). The inherent weakness of this method of analysis is that the digestion of nitrogen-free extract (NFE) which is presumed to represent the more digestible carbohydrates, is found by difference, from the results of the other determinations. Consequently, the digestion of carbohydrates calculated in this manner include any and all experimental errors (Van Soest, 1969). The basic problem associated with this technique appears to be the failure of crude fibre and NFE estimates to indicate a nutritional separation of fibre. To elaborate, there is a failure to clearly define the nutritional availability of carbohydrates; thus separating lignin from the soluble digestible carbohydrates (Van Soest, 1969). Fonnesbeck (1969) suggests that the soluble carbohydrate fraction should in turn be divided into: (1) fibrous carbohydrate, digestible by symbiotic microorganisms in the digestive tracts of herbivorous animals; and (2) soluble carbohydrate, digested by enzymes secreted by the digestive system of all animals. Other work by Fonnesbeck et al, (1967, 1968 b) suggests that there is indeed a significant difference in nutritive values associated with the different carbohydrate fractions. They further suggest that the analysis which provides the most meaningful separation of nutrients is the estimate of cell-wall constituents (CWC) contained within the plant, (Fonnesbeck, 1968 b).

A review of the literature regarding recent progress in the field of equine nutrition points to the need for additional research. It was for the purpose of increasing the amount of information pertaining to the nutrient requirements of horses, that the following experiments were employed.

EXPERIMENTAL

Reproductive Experimental

Objectives

This experiment was designed to monitor several performance parameters in order to judge the relative adequacy of recommended nutrient requirement estimates, based on NAS-NRC, Nutrient Requirements of Horses (1973), for pregnant and lactating mares under central Alberta winter conditions. The parameters used included weights of the mares throughout gestation and early lactation, blood serum chemistry profile of mares and foals, foal birth weights and rate of gain, and analysis of several constituents of mares' colostrum and milk.

Animals

Working with twelve half Arabian mares (half sibs) leased from Mr. B. Langdale of South Edmonton, this experiment was initiated in December 1974 and terminated in June, 1975. Horses were cared for by the students of the Horse Husbandry course under the supervision of Mr. W. D. Ratcliff, Instructor, Department of Animal Science, Olds College , Olds, Alberta.

Monthly maximum and minimum temperatures were recorded for the duration of the experiment. This data together with temperatures from 1974 to 1964 are presented in Table 7.

Table 7. Monthly Average Wind Speed and Monthly Maximum and Minimum Temperatures for Central Alberta [1]

Month	Year	Wind(mph)[2]	Temperature[3] Min.	Temperature[3] Max.
Jan.	1970	7.9	-47.8	41.3
	1971	8.3	-37.5	44.7
	1972	9.1	-54.8	43.0
	1973	7.8	-28.9	43.0
	1974	8.8	-44.0	35.3
	1975	-	-34.0	41.0
Feb.	1970	8.1	-10.7	41.9
	1971	7.3	-28.0	44.0
	1972	7.1	-28.1	34.9
	1973	8.1	-26.1	48.8
	1974	8.6	-33.4	40.9
	1975	-	-25.0	49.0
March	1970	9.3	-23.2	46.3
	1971	8,8	-20.3	39.9
	1972	7.2	-44.3	46.7
	1973	7.4	- 0.1	52.5
	1974	8.1	-26.4	40.8
	1975	-	- 5.0	45.0
April	1970	8.0	16.0	59.8
	1971	10.1	- 0.9	62.2
	1972	9.9	5.1	79.2
	1973	8.4	11.7	65.0
	1974	8.3	7.3	71.8
	1975	-	-7.0	60.0
May	1970	11.2	23.1	83.9
	1971	9.2	26.9	85.0
	1972	7.7	25.3	85.6
	1973	10.7	27.8	81.6
	1974	7.7	26.8	74.3
	1975	-	23.0	70.0
June	1970	8.8	37.0	94.2
	1971	9.4	37.2	82.1
	1972	8.7	36.1	81.2
	1973	9.5	36.0	87.5
	1974	8.1	38.0	83.3
	1975	-	-	-

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[1] Temperatures prior to 1975 were recorded at the Edmonton International Airport.

[2] For 1975 wind speeds were not available.

[3] Temperatures for 1975 were recorded at Olds College.

The trial was initiated by the random allotment of the twelve mares into two groups. Then adjustments were made based on predicted foaling dates, in an effort to insure that both groups were due to foal at similar times. Mares were identified by tags attached to their halters. Both groups of mares were then treated for parasites by esophogeal administration of Equisol (Merk, Sharpe and Dohme Ltd.).

The first group of mares were fed a diet designed to meet NRC requirement levels (NAS-NRC, Nutrient Requirements of Horses, 1973). The second group received the same diet at a 15% lower level of daily intake. Iodized salt and ground limestone were offered free choice for the duration of the experiment.

All mares were contained in an open pen with dimensions of approximately 100 X 30 meters. They were individually fed in tie stalls and received no other shelter except shortly before and after parturition, when they were housed in a heated barn, approximately 5 C. When a mare showed signs of imminent parturition, she was moved into a large stall and observed continually until foaling. No assistance was given at foaling.

After parturition, placental weights were recorded. An iodine navel-dip and merconial enemas (Fleet) were applied to foals. Foals were also encouraged to suckle before any colostrum samples were taken.

Within twelve hours, where possible, colostrum samples were taken from the mare and blood samples were taken from the foal. Additional blood samples were taken from the foal for three more consecutive days and again 30 days later at the termination of the trial. A final milk sample was also taken at the termination of the trial.

Experimental Diets

The mares were maintained through three nutritional phases (early gestation, late gestation and early lactation), necessitating periodic adjustments in diet. Formulation and composition of experimental diets are given in Table 8. The experimental design outlining dietary treatment is summarized in Table 9. Since it was not the purpose of this experiment to introduce new feedstuffs for horses, the selection of ingredients was limited to what might be considered common components of horse rations in central Alberta. Major dietary ingredients were timothy hay (*Phleum pratense*); barley (*Hordeum vulgare*) and oats (*Avena sativa*).

Collection of Data

Mares were weighed at the middle of every month and blood samples were taken at that time. A final weight was recorded and blood samples taken at the termination of the experiment. As previously described, colostrum and milk samples were taken at 12 h and 30 d

Table 8. Formulation and Composition of Diets: Mares

Diet:	Phase I (Early Gestation)	Phase II (Late Gestation)	Phase III (Early Lactation)
Ingredients %			
Timothy	39.0	37.1	36.0
Oats	26.0	32.3	31.3
Barley	34.0	24.7	24.0
Soybean Meal (45.8% protein)	-	4.9	7.9
Ground Limestone	0.5	0.5	0.48
Iodized Salt	0.5	0.5	0.48
Composition (analyzed)			
Crude Protein (g/kg)	92.9	116.9	125.4
Digestible Energy (Meal/kg)	2.76	2.8	2.8
Composition (Calculated)			
Calcium (g/kg)	5.2	4.8	5.2
Phosporus (g/kg)	2.4	4.0	2.8

Table 9. Dietary Treatments: Mares

Mare #	Phase I December 16-February 19 kg-feed/day	Phase II Feb.19-foaling kg-feed/day	Phase III kg-feed/day
Group 1[1]			
49	5.0	open[2]	--
59	6.1	5.6	--
69	7.9	7.0	11.1
79	6.2	5.7	9.2
89	6.6	6.3	10.2
99	6.0	open	--
Group 2			
204	6.6	6.3	10.2
205	5.9	open	--
206	7.1	open	--
207	5.6	6.9	10.2
208	7.0	6.8	10.5
209	6.8	6.6	9.1

[1] The same diets were used for both treatment (See Table 8).

[2] Mares which were pregnancy tested and found not pregnant were classified as "open" and maintained at Phase I level.

after parturition, respectively. Foal birth weights and 30d weights were recorded. Blood samples from foals were collected in a manner previously described. Fecal samples from mares for calculation of energy and nitrogen digestibilities were also taken.

The objective of the weight measurements were to monitor relative weight changes as they related to the nutritional adequacy of the two rations. Based on the assumption that random allotment of mares to treatments would result in response in weight gain or loss being due to treatment effect, (Steele and Torrie, 1960); further assumption was made, that if initial mean weights of the mares were similar, the mean weights of foals at birth, would also be similar, if there was no treatment effect. Conversely, if there were treatment effects, these would be reflected in the weights of the foals at birth and at one month.

Analysis of Feed, Blood and Milk

The energy and protein content of the feed ingredients was analyzed from samples submitted to the Soil and Feed Testing Laboratory, Alberta Agriculture, O.S. Longman Building, Edmonton, Alberta.

The chemical analyses of blood plasma samples were done at Hanson and Associates Medical Laboratories in a manner described by A.O.A.C. (1965).

The purpose of this facet of measurement is (1) to gain further knowledge and appreciation of the variation of some blood parameters and, (2) to enable us to compare these results with similar data obtained elsewhere and, (3) to enable us to compare these parameters in light of the effects of nutritional standard between treatments.

The analyses of colostrum and milk samples from mares were conducted at the Soil and Feed Testing Laboratories, O.S. Longman Building, Edmonton, Alberta. The procedures used for analysis were as follows: calcium and phosphorus, atomic absorption spectrophotometry; lactose, A.O.A.C. No. 16.049 and No. 16050; lipids, Babcock A.O.A.C. No. 16.053; and proteins, Kjeldahl (A.O.A.C. 1965).

Digestibility Study with Mares

Estimates of digestibility of energy (D.E.) and nitrogen (D.N.) were made during the late gestation phase for the eight pregnant mares and at the same time for the remaining four open mares. A seven day total collection period was involved. In addition, the random grab method was used (McCarthy et al 1974 and Vogtmann et al 1975). Random grab samples were taken at 10:00 - 10:30 A.M. and 5:00 - 5:30 P.M. every day of the collection period. These samples were frozen and then transported to the University of Alberta and dried at 65 C for 72 hours. Feed samples were also returned to the University laboratory and dried. After being allowed to dry, all samples were finely ground in a Christie and

Norris feed grinding mill. Analyses of total energy, total Kjeldahl nitrogen and dry matter were made according to the methods of A.O.A.C. (1965). Energy content of feed and fecal samples were determined with a Parr Oxygen Bomb Calorimeter. The determination of Kjeldahl nitrogen involved the use of a commercial 'Kel-pack' as a catalyst and 4% boric acid for the collection of ammonia.

The digestibility of the diets was estimated using the insoluble ash method of McCarthey et al (1974). Feed and fecal samples were boiled in 4N-HCl for 0.5 hours. The resulting insoluble ash residue was then used as an internal indicator from which a digestibility coefficient was calculated.

Digestibility Experiment

Objectives

This experiment was designed to compare the reliability of the 4N-HCl insoluble ash technique with the total fecal collection method, for determining apparent digestibility of nitrogen and energy in horse diets.

Animals and Diets

A total of seven different geldings were used in a series of three trials which were conducted over the period from April, 1976 to March, 1976. Experimental work was conducted at the Edmonton Research Station, University of Alberta, Edmonton, Alberta. Experimental design outlining the diets fed for the experiments are presented in Table 11. Formulation and composition of diets are given in Table 10. The diet used for the first collection period was the same as that used with the mares at Olds College.

Methods and Procedure

(a) Rate of Passage

Four geldings were placed on a diet (Table 10) three weeks prior to the initiation of the trial. After this period of adjustment, cotton cloth strips 5.0 X 2.0 cm were placed in the feed. This method of estimating rate of passage is not based on any previous use of cotton strips and is therefore, to my knowledge, an original method of estimation. Previous use of cotton thread was made by Balch and Johnson (1950) as an indicator of cellulolytic activity in cattle and horses and not as a direct estimate of rate of passage of digesta.

Twenty-five cotton strips were added to the ration for each horse on trial. Separation of the strips was prevented by feeding the ration as a mash, for that day only. After 48h, fecal material was collected every 4h for the first replicate and every 2h for the second. The material was then strained and the first evidence of cloth noted. The first replicate was begun May 28 and terminated May 31, 1975 while the second was begun June 7 and terminated June 10, 1975.

Table 10. Formulation and Composition of Diets: Geldings.

Diet:	Trial 1	Trial 2	Trial 3
Ingredients %			
Timothy	37.1	--	--
Oats	32.3	72.2	72.2
Barley	24.7	--	--
Soybean Meal (45.8%)	4.9	--	--
Dehydrated Alfalfa (17.0%)	--	27.3	27.3
Ground Limestone	0.5	--	--
Iodized Salt	0.5	0.5	0.5
Composition (analyzed)			
Digestible energy (Meal/kg)	2.8	2.8	2.8
Crude Protein (g/kg)	117.0	122.2	122.2
Composition (Calculated)			
Calcium (g/kg)	5.4	4.3	4.3
Phosphorus (g/kg)	4.1	3.1	3.1

Table 11. Dietary Treatments: Geldings

Horse	Trial 1 (kg-feed/day)	Trial 2	Trial 3
1	4.4	6.9	6.0
2	4.1	5.1	5.1
3	4.4	--	--
4	5.5	6.9	--
5	--	6.1	--
6	--	--	5.1
7	--	--	6.0

(b) Digestibility Studies

A total of three digestibility trials were completed using four geldings for each trial. Seven geldings in all were used over the three trials. One gelding used for all three trials was a two-year-old. Of the remaining six, three were over 12 years old and three were over 5 years old.

Horses for these trials were housed in a heated barn and separated by tie stalls, approximately 3.2 X 2.0 meters in size. The levels of feeding are described in Table 11.

Trial 1, using the diet obtained from Olds College, involved two replicates using four geldings. Fecal collections were taken from a concrete floor, twice daily. After the second collection each day, the floor was thoroughly washed. As a result, it is probable that some small portions of fecal material were lost. The collection period lasted seven days. Consequently, all the horses tended to have sore feet by the end of the collection period. A recovery period of seven days was allowed between the replicates with the animals being stabled in a dirt pen.

Trial 2, using the ration shown in Table 10, involved two replications with the objectives of comparing the effects of days of sampling on random grab sample results. Based on the results of trial 1 and the rate of passage experiment, the collection periods were shortened to 4 days. Otherwise this trial was completed in a manner similar to trial 1.

Trial 3 was started seven months later in January, 1976. Four geldings were individually housed in metabolism crates. The crates themselves were constructed of wood with slotted floors in the middle section to allow for separation of urine and feces. These crates measured approximately 2.5 X 1.5 meters. Thus horses were allowed only minimal movement. Fecal samples were collected in the manner described in trial 1, except that feces were taken from the metabolism crates rather than from the concrete floor. A total of five replicates were completed and the trial was terminated March 1, 1976. Analyses of feed and fecal samples were completed in the manner described in the Reproductive Experiment.

Statistical Methods

Reproductive Experiment

Using the parameters measured and described earlier, one-way analyses of variance were computed to determine if significant differences existed between treatments. Ration treatment was considered to be the only fixed source of variation. Notations used to indicate level of significance are: $^{*}(P < 0.05)$ and $^{\dagger}(P < 0.10)$.

The parameters in this experiment were measured over a lengthy period of time. As a result, it was decided to plot these variables against time, some of which are presented in Appendix A, in order to more clearly establish the presence or absence of any trends.

Digestibility Studies - Mares

The potential comparison presented by estimates of digestibility coefficients from the insoluble ash technique were the relationships of apparent digestibility vs. time and apparent digestibility of open vs. pregnant mares. One-way analyses of variance were calculated for these relationships.

Digestibility Studies - Geldings

Multiway classifications (Steele and Torrie, 1960) with two-way analyses of variance were done for trials 1 and 2. One-way analyses of variance were done for trial 3 as time period was not a variable factor. In addition to multiway classifications for trial 1, a comparison of grand means using a t test and pair wise correlations of insoluble ash vs. total collection techniques were calculated for trial 3.

RESULTS AND DISCUSSION

This discussion pertains to the two experiments; the reproductive study and the digestibility studies. The results for the former are considered under the following catagories:

(a) performance

(b) digestibility of diets

(c) blood constituents

Results of the reproductive experiment are discussed in terms of mare and foal weights, colostrum and milk samples, and mare and foal blood serum constituents. Comparisons for digestibility of diets are discussed and reference to weather conditions are made.

Results of the digestibility experiment are discussed in terms of insoluble ash and total collection methods of estimating apparent digestibilities of feeds. Rate of passage of feed is considered in light of its effect on collection periods.

Reproductive Study

Performance

(a) Weights

Weights of mares (Table 12) were recorded for the duration of the trial and weights of foals (Table 13)

Table 12. Record of Weights for Mares.

Mare	Period [1]						
	Dec.	Jan.	Feb.	Mar.	April	May	June
Group 1							
49	418	386	336	349	355	347	-
59	445	468	418	443	450	455	476
69	600	611	557	576	522	527	--
79	459	477	448	464	486	436	--
89	495	509	468	488	495	486	439
99	464	455	400	419	434	439	--
Mean	480	484	437	456	437	431	469
Group 2							
204	500	507	464	479	493	486	436
205	468	464	418	414	427	432	--
206	384	427	375	412	402	409	--
207	541	548	495	506	511	489	431
208	527	536	500	500	507	506	450
209	513	523	477	518	486	432	436
Mean	489	501	454	471	471	459	434

---

[1] Weights were recorded at monthly intervals commencing December 16, 1974.

Table 13. Record of Weights for Foals

Foal	Birth Weight	30-day Weight	Average Daily Gain (ADG)
F-69	53.6	87.2	1.12
F-79	43.6	84.0	1.35
F-89	51.8	80.0	0.94
Mean	49.7	83.7	1.13
F-204	45.9	75.0	0.97
F-207	44.5	79.6	1.17
F-208	55.9	81.0	0.84
F-209	49.1	83.0	1.13
Mean	48.9	79.7	1.03

were recorded at birth and at one month of age. No significant differences were found due to treatment between either mares or foals.

Mean weights of 462.4 kg for mares is well within the suggested range of 400-600 kg (Ensminger, 1969) for light horses. Mean weights of 49.3 kg for foals at birth are similar to birth weights of 110 lb. (50 kg) for 1,071 trotting-bred foals (Morrison, 1946).

(b) Rates of Gain

Mean average daily gain (ADG) for foals, at 30 days of 1.07 kg per day is within the range of estimated growth curves cited by NAS-NRC,(1973). The calculation of ADG for foals Table 14 shows no significant difference between groups. This would appear to imply that while some significant differences were noted among milk and blood constituents, to be reported later, these differences were not reflected in the growth performance of the foals over the period of time that these measurements were taken. However, it should be pointed out that since these weight measurements were only taken up to one month after foaling, several important effects may have been missed. This experiment was terminated somewhat prematurely for reasons important to the owner of the mares.

Mares generally attain maximum milk production at approximately 8 weeks (NAS-NRC, 1973). Had this experiment been allowed to continue beyond this peak period we may have seen some trends develop, in terms of foal performance. For example, it is logical to suggest that differences in nutritional levels of the two rations would be most noticeable after the maximum period of milk production had been reached. Furthermore, a nutritional deficiency has, by nature, a cumulative effect; that is, that unless corrected, a nutritional deficiency of any kind tends to become more critical with the passage of time. Consequently, the lack of significant differences found between treatments, particularly with regard to foal weights, cannot be assumed to prove that NRC guidelines are in excess of the true minimum requirements for all pregnant and lactating horses over the whole period of lactation.

One parameter that was not measured was that of conception rate for mares. This parameter, in particular, is of great economic importance to horse breeders. This is especially true in light of the fact that conception rates in horses, relative to other domestic species, is historically low (Ensminger, 1969) and the effects of flushing on conception has not been widely studied.

Weather

The effects of weather conditions, particularly ambient temperature, on nutritional requirements has been well studied with a number of species, (Ensminger, 1969). The fact that NAS-NRC guidelines (1973) make no particular reference to these effects suggests that there may be considerable variety in terms of relative adequacy of these guidelines under varying weather conditions.

Alberta's relatively inclement winters provide an excellent opportunity to study the adequacy of NAS-NRC (1973) guidelines in animals which are frequently subjected to cold stress conditions during the course of the season. Presumably, if no significant differences are noted between treatments for these mares, no significant differences are likely to be forthcoming in more favorable climates.

Weather data for 1970 through June, 1975 are presented in Table 7. As can be seen from this data, the weather conditions for duration of this trial were somewhat milder than normal. Nevertheless, there were frequent periods when the mares were subjected to considerable cold stress.

Table 14, taken from Young (1975) indicates the variable levels of cold stress due to wind velocity and ambient temperatures.

Colostrum and Milk

Analyses of variance for levels of fat, lactose, calcium and phosphorus in colostrum and milk Table 15, revealed no significant differences due to

Table 14. Daily Metabolizable Energy Intake as it Relates to Ambient Temperatures

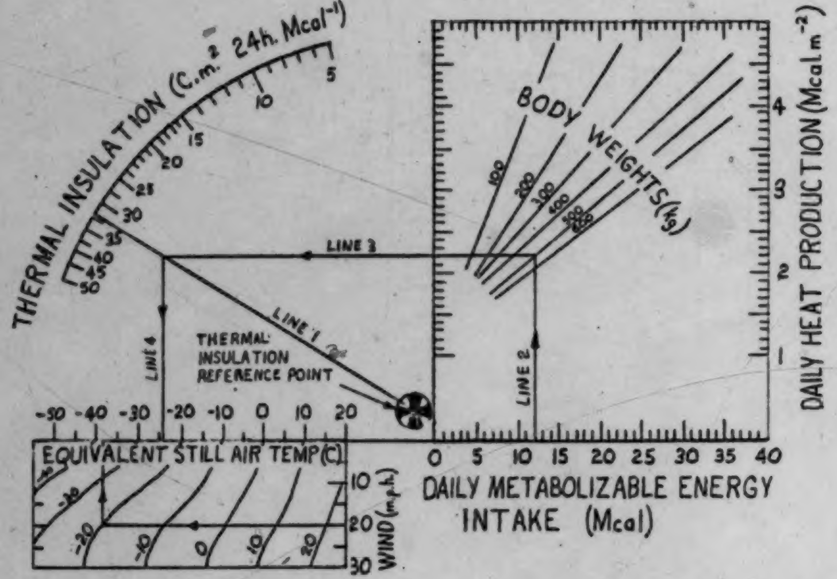
THERMAL INSULATION (C.m² 24h. Mcal⁻¹)
5
10
15
20
25
30
35
40
45
50
BODY WEIGHTS (kg)
100
200
300
400
500
600
DAILY HEAT PRODUCTION (Mcal m⁻²)
1
2
3
4
LINE 1
LINE 2
LINE 3
LINE 4
THERMAL INSULATION REFERENCE POINT
-50
-40
-30
-20
-10
0
10
20
EQUIVALENT STILL AIR TEMP (C)
WIND (m.p.h.)
10
20
30
0
5
10
15
20
25
30
35
40
DAILY METABOLIZABLE ENERGY INTAKE (Mcal)

Table 15. Analyses of Mares' Milk

		Ingredient %				
Mare	Sample[1]	Fat	Protein	Lactose	Calcium	Phosphorus
69	A	--	--	--	--	--
	B	1.25	1.73	7.87	0.11	0.05
79	A	1.20	7.79	5.12	--	--
	B	0.75	1.93	7.92	0.13	0.05
89	A	--	--	--	--	--
	B	0.80	1.68	7.12	0.11	0.04
Mean	A	1.20	7.79	5.12	--	--
	B	0.85	1.78	7.64	0.12	0.05
204	A	0.70	12.82	4.80	0.08	0.07
	B	0.70	2.27	7.85	0.10	0.05
207	A	2.40	3.74	6.78	0.12	0.08
	B	1.25	2.25	7.80	0.12	0.07
208	A	1.05	19.25	3.38	0.12	0.09
	B	0.60	2.44	7.14	0.10	0.06
209	A	1.30	8.41	5.64	0.08	0.10
	B	0.55	2.27	7.80	0.08	0.06
Mean	A	1.36	11.55	5.13	0.10	0.09
	B	0.77	2.31	7.65	0.10	0.06

---

[1] Two samples were taken from each mare. The first (A) was taken within 12 hours after parturition. The second was taken 30 days after parturition.

Table 16. Correlations of Insoluble Ash vs. Total Collection for Digestible Nitrogen and Energy - Reproduction Study

Digestible Nitrogen:

Total Collection			Insoluble Ash		
Collection Period:					
3 day	5 day	7 day	3 day	5 day	7 day
-	0.72	0.82	0.97	0.97	0.92
	-	0.92	0.65	0.84	0.82
		-	0.73	0.87	0.86
			-	0.93	0.83
				-	0.93
					-

Digestible Energy:

-	0.82	0.90	0.97	0.97	0.97
	-	0.94	0.79	0.85	0.87
		-	0.87	0.90	0.90
			-	0.96	0.96
				-	0.98
					-

treatment. Milk protein samples were found to be significantly ($P < 0.05$) higher in group 2 as compared to group 1 mares. Overall means for colostrum and milk constituents are similar although slightly higher in protein and lower in fat than findings of Ensminger (1969) and Morrison (1946).

Since foals were given priority for colostrum it was not possible to collect sufficient colostrum samples to provide for analysis for a number of mares. Consequently, interpretations derived from these results should be approached with a certain amount of reservation.

Digestibility of Diets

Digestibility trials conducted at Olds College were initially begun in order to find differences in the ability to digest feeds on the part of the two groups of mares. However, the lack of available facilities dictated that total collections be made from a clay floor, which I feel introduced substantial error in our findings. Anticipating that these difficulties may arise, it was decided to take random grab samples simultaneously with total collections, as a sub-sample of total collections for the determination of apparent digestibilities using the 4N-HCl insoluble ash method (McCarthy et al 1974).

In spite of the error associated with total collections for these mares, correlations (Table 16) DN and DE suggest a linear relationship between the two methods of determination. Analyses of the data, Table 17&18 indicated significant differences ($P < 0.05$)between methods for

Table 17. Apparent Digestibilities of Nitrogen and Energy for Group 1 Mares

Mare	3 day Total	5 day Total	7 day Total	Mean	SD*	3 day HCL	5 day HCL	7 day HCL	Mean	SD*
					Digestible Nitrogen (%)					
49	66.2	69.9	70.7	68.9±	1.39	87.7	84.4	88.7	86.9	1.30
59	77.7	81.0	78.5	79.1	0.99	86.4	86.8	85.7	86.3	0.32
69	77.2	80.9	79.1	79.1	1.07	85.0	84.3	83.7	84.3	0.38
79	60.9	66.4	65.3	64.2	1.68	87.8	85.9	84.6	86.1	0.93
89	77.2	78.8	78.8	78.3	0.53	87.9	86.9	88.5	87.8	0.47
99	77.1	75.0	75.0	75.7	0.70	87.0	84.5	88.2	86.6	1.09
Mean	72.7	75.3	74.6			87.0	85.5	86.6		
					Digestible Energy (%)					
49	57.5	59.7	63.7	58.6	3.28	82.7	79.1	85.9	82.6	1.96
59	66.3	70.9	71.6	69.6	1.66	79.4	79.9	81.1	80.1	0.50
69	69.4	74.0	74.1	72.5	1.55	79.9	78.8	79.7	79.5	0.34
79	44.8	57.3	56.8	53.0	4.08	82.7	82.0	80.4	81.7	0.68
89	69.1	70.3	70.0	69.8	0.36	83.6	81.7	83.8	83.0	0.67
99	63.2	65.9	65.8	65.0	0.88	79.1	78.9	83.8	80.6	1.60
Mean	60.9	66.4	67.0			81.2	80.1	82.5		

---

* Standard deviation of mean across collection periods.

Table 18. Apparent Digestibilities of Nitrogen and Energy for Group 2 Mares.

Mare	3 day Total	5 day Total	7 day Total	Mean	SD*	3 day HCL	5 day HCL	7 day HCL	Mean	SD*
				Digestible Nitrogen (%)						
204	72.5	76.6	76.0	75.0	1.28	88.0	86.4	87.8	87.4	0.50
205	64.0	72.5	72.4	69.6	2.81	88.5	87.6	88.9	88.3	0.38
206	76.3	77.3	74.9	76.2	0.70	86.1	84.4	87.6	86.0	0.92
207	68.9	76.9	79.1	75.0	3.10	85.6	88.2	88.4	87.4	0.90
208	76.3	80.8	81.1	79.4	1.55	87.3	85.7	88.3	87.1	0.76
209	75.4	74.4	76.6	75.5	0.64	88.9	84.6	84.9	86.1	1.39
Mean	72.7	76.4	76.7			87.4	86.2	87.7		
				Digestible Energy (%)						
204	60.6	66.6	66.9	64.7	2.07	82.8	80.6	83.3	82.2	0.83
205	55.9	60.0	62.8	59.6	2.00	84.6	82.0	85.1	83.9	0.96
206	69.3	72.7	70.1	70.7	1.03	82.0	81.2	85.2	82.8	1.22
207	53.8	64.9	69.5	62.7	4.66	83.7	82.0	83.1	82.9	0.50
208	68.3	73.7	74.2	72.1	1.89	82.9	80.3	84.0	82.4	1.10
209	63.3	67.7	71.0	67.3	2.23	83.4	80.5	81.3	81.7	0.86
Mean	62.7	67.6	69.1			83.2	81.1	83.7		

* Standard deviation of mean across collection periods.

determination of digestibility coefficients for protein and energy, and between individual animals. However, no significant differences were found due to treatment effect, pregnancy or between the three time periods (3, 5 and 7 days) for which collections were made.

These results were found on the basis of either method of determination. However, since the variation within groups was large using the total collection method, the error term used for analysis of variance was also large, resulting in lower F values than would have been found had the total collection provided more consistent results. Therefore, the possibility that significant differences due to treatment effect were missed by the total collection method, increases the dependency on the insoluble ash technique. This technique has not yet been shown to be reliable for horses. Consequently, it cannot be assumed that there were no significant differences in digestibilities between mares, based solely on the insoluble ash technique. Therefore, the reliability of the insoluble ash technique for horses must first be established before comparisons between groups of mares can be made. It was because of this possibility that digestibility studies were done at the Edmonton Research Station in order to compare the insoluble ash method with the total collection method under more controlled conditions. The findings of these studies will be discussed later. For the present, it will be assumed that energy and nitrogen digestibility differences due to treatment or stage of pregnancy were not encountered.

Analysis of the digestibility data (Table 17 and 18) revealed that the standard deviations across collection periods were generally much larger for the total collections than for the random-grab samples for insoluble ash. This substantiates the impression that significant sampling error was involved for total collections, and that much of this sampling error was avoided by the use of the insoluble ash technique.

Blood Serum Constituents

(a) Calcium (Ca) and Phosphorus (P)

No significant differences were noted for serum levels of Ca and P (Tables 19 and 20) between groups. Serum levels of Ca were also similar for mares and foals as well as pregnant and non-pregnant mares. However, serum P levels in foals were consistently higher than serum P levels in either pregnant or open mares. This is apparently associated with the greater amount of bone growth in foals and is in general agreement with Wolff et al (1969). Both serum Ca and P levels for mares are in close agreement with results of kirkham et al (1969) who reported mean levels for Ca, 11.76 mg%; and P, 3.36 mg%.

(b) Glucose

No significant differences were found in the measurements of plasma glucose. Blood serum concentrations of glucose are affected by the relative state of fast

(Alexander, 1954) associated with the individual tested. Since no attempt was made to take these samples under fasting conditions, serum glucose levels are subject to considerable variation, as can be seen in Tables 19 and 20. As a result of the variation encountered, interpretation of results is difficult. In spite of this, these results are in general agreement with the range shown by Alexander (1954) of approximately 80 - 150 mg/100 ml.

(c) Albumin (Alb) and Uric Acid (U.A.)

Concentrations of both plasma albumin and uric acid showed no significant differences between groups of mares or foals. Furthermore, these concentrations did not appear to be affected by pregnancy or stage of maturity. Mean value of 3.1 g/100 ml for plasma albumin in mares is in general agreement with Wolff et al (1969) of 2.3 - 3.8g/100 ml for plasma albumin.

(d) Blood Urea Nitrogen (BUN)

The measurement of BUN is often used to estimate efficiency of kidney function in humans. In cases of acute renal failure the BUN will rapidly rise to toxic levels. In horses, there is evidence (Medway,(1969) and Wolff et al, (1969) that normal BUN concentrations can range from approximately 5 - 40 mg/100ml. None of the horses used in this experiment showed any evidence of renal failure. BUN levels, Tables 19 and 20, were all

Table 19. Blood Chemistry Profiles for Group 1 Mares

Mare #	Ca (mg/dl)	P (mg/dl)	Gluc (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)	Chol (mg/dl)	TP (gm/dl)	Alb (gm/dl)	Bili (mg/dl)	Alk-P (mU/dl)	LDH (mU/dl)	SGOT (mU/dl)
						December						
49	10.8	4.2	89	17	0.6	72	7.7	2.8	0.65	216	318	320
59	11.1	3.6	89	17	0.5	95	7.3	2.9	0.78	218	310	370
69	11.4	3.6	93	19	0.6	105	7.9	2.9	1.04	129	261	340
79	11.9	2.9	95	20	0.5	108	7.5	3.0	0.98	181	243	350
89	11.2	4.3	95	17	0.6	121	7.1	3.1	0.99	222	311	360
99	11.7	4.3	84	20	0.6	73	7.6	2.8	0.59	155	323	320
						January						
49	12.4	4.6	104	15	0.9	102	7.4	2.9	0.78	156	363	360
59	12.3	4.0	105	14	0.5	113	7.6	3.2	1.06	171	266	376
69	12.7	3.8	95	18	0.5	114	7.6	3.0	0.93	103	234	340
79	12.8	3.3	95	17	0.5	113	7.2	3.2	0.91	135	230	350
89	12.7	3.9	98	14	0.5	112	7.6	3.2	0.95	188	290	346
99	12.3	4.3	90	16	0.4	114	7.4	3.8	0.76	238	239	360
						February						
49	12.4	3.3	144	14	0.4	109	7.3	3.1	1.09	180	319	342
59	11.5	2.8	151	13	0.4	85	7.4	3.1	1.35	175	275	310
69	10.1	3.1	125	14	0.4	101	7.7	3.0	1.62	135	295	280
79	12.9	2.6	160	17	0.4	115	7.9	3.4	1.22	172	286	365
89	11.3	2.6	160	11	0.4	87	7.3	2.9	1.26	222	359	340
99	12.1	2.5	155	13	0.3	100	7.7	2.8	1.04	214	244	320
						March						
49	11.8	3.9	92	13	0.7	104	7.0	2.9	0.90	192	313	330
59	11.4	3.4	102	13	0.9	100	7.3	3.0	1.08	161	279	350
69	11.4	3.2	117	15	0.8	114	7.4	3.0	1.20	123	229	310
79	12.3	3.1	109	15	0.9	113	7.3	3.2	1.06	155	215	320
89	11.7	3.9	89	14	0.8	106	7.7	3.2	1.06	214	254	320
99	11.4	3.6	109	14	0.7	113	7.0	2.7	0.90	211	246	340
						April						
49	12.8	4.0	91	12	0.8	116	7.3	2.9	1.17	234	346	226
59	12.1	3.5	94	13	0.8	104	7.8	3.1	1.26	181	281	300
69	12.4	2.6	125	9	0.9	123	7.7	3.0	1.25	171	311	360
79	12.0	2.9	99	14	0.9	114	7.1	3.0	1.54	164	215	250
89	12.0	3.0	109	14	0.9	115	8.4	3.0	1.52	292	254	255
99	11.8	3.4	82	14	0.9	133	7.5	2.8	1.20	227	263	320
						May						
49	11.5	4.1	63	12	0.5	115	6.8	2.8	1.12	241	419	304
59	11.5	2.9	106	13	0.6	91	6.8	2.6	1.20	197	304	290
69	12.5	3.1	86	12	0.6	102	7.1	2.5	1.12	162	313	292
79	*12.5	3.5	150	13	0.7	152	6.9	3.0	1.26	550	314	280
89	11.1	3.0	64	11	0.5	124	7.7	3.0	2.28	480	418	430
99	11.9	4.0	59	14	0.7	118	6.9	2.6	1.00	234	290	296
						June						
49[1]	--	-	-	-	-	-	-	-	-	-	-	-
59[2]	-	-	-	-	-	-	-	-	-	-	-	-
69[3]	-	-	-	-	-	-	-	-	-	-	-	-
79	11.7	2.8	158	15	0.7	108	6.5	2.8	1.35	345	324	290
89	10.9	1.8	106	13	0.6	82	7.7	3.0	1.09	331	328	298
99[4]	-	-	-	-	-	-	-	-	-	-	-	-

*79 Sample taken May 6.

[1] Terminated at the end of May - open mare.

[2] Terminated at the end of May - not yet foaled.

[3] Terminated at the end of May - finished trial.

Table 20, Blood Chemistry Profiles for Group 2 Mares

Mare #	Ca (mg/dl)	P (mg/dl)	Gluc (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)	Chol (mg/dl)	TP (gm/dl)	Alb (gm/dl)	Bili (mg/dl)	Alk-P (mJ/dl)	LDH (mU/dl)	SGOT (mU/dl)
						December						
204	11.4	3.5	82	14	0.5	87	7.9	2.5	0.68	185	280	376
205	11.5	3.5	91	17	0.5	85	7.8	3.1	0.90	139	294	340
206	10.6	4.3	91	17	0.5	81	7.5	2.8	0.61	169	313	300
207	10.8	4.1	87	19	0.6	105	7.0	2.7	0.74	217	310	360
208	10.9	4.0	95	18	0.7	90	7.4	3.1	1.09	183	295	324
209	10.5	3.7	89	18	0.6	86	7.0	3.0	1.30	285	270	280
						January						
204	12.1	4.1	91	17	0.5	107	7.3	3.3	1.20	240	267	316
205	12.8	4.0	103	13	0.5	132	7.1	3.4	0.83	177	296	404
206	12.2	4.4	92	12	0.5	101	7.7	2.6	0.82	150	273	314
207	12.3	3.9	94	17	0.6	124	7.4	3.3	1.18	153	253	340
208	12.2	3.8	94	15	0.6	109	7.4	3.0	0.92	127	232	330
209	12.9	3.8	86	15	0.6	100	7.8	3.0	0.76	135	309	358
						February						
204	12.0	3.6	164	14	0.4	87	7.2	3.3	1.80	235	325	320
205	12.2	3.2	201	16	0.5	134	7.5	3.5	2.34	188	279	390
206	12.0	2.7	187	10	0.4	77	8.0	2.7	0.85	183	322	280
207	12.1	3.6	248	16	0.5	112	7.3	3.2	2.19	182	233	300
208	12.1	2.6	169	14	0.4	99	7.4	3.0	1.43	129	233	302
209	10.5	2.7	181	15	0.5	103	7.2	2.8	0.90	150	268	240
						March						
204	11.7	3.3	74	14	0.9	101	7.2	3.4	1.34	236	330	320
205	11.8	3.0	99	15	0.8	121	6.8	3.3	1.04	192	233	350
206	11.8	3.8	99	12	0.8	106	7.7	2.8	0.87	156	314	310
207	12.1	3.8	97	15	1.0	129	7.4	3.4	1.59	192	312	340
208	11.6	3.1	93	12	0.7	113	7.0	3.0	1.12	121	191	310
209	10.6	3.4	91	16	0.9	122	7.2	2.9	1.19	145	228	320
						April						
204	12.2	3.3	86	15	0.9	108	7.2	3.3	2.31	231	253	265
205	12.9	3.3	112	12	0.7	139	7.3	3.4	1.27	214	271	340
206	12.7	3.8	80	11	1.0	111	8.3	2.6	1.02	250	284	265
207	12.2	3.7	133	13	0.8	138	7.5	3.3	2.28	187	243	270
208	13.0	3.2	98	11	0.8	134	7.4	3.1	1.65	158	208	370
209	11.9	3.1	99	14	0.8	107	7.0	2.8	1.27	176	231	250
						May						
204	12.1	4.3	72	13	0.8	134	6.8	3.1	2.85	390	320	375
205	12.6	4.0	79	13	0.7	121	6.9	3.0	1.26	268	371	350
206	11.8	3.4	86	12	0.7	94	7.1	2.4	1.09	235	332	270
207	10.5	2.7	99	15	0.8	113	6.7	2.7	1.18	171	236	255
208	11.3	2.7	136	11	0.7	142	6.5	2.7	1.90	193	269	254
209	11.9	3.8	83	13	0.7	157	7.1	3.0	2.12	256	338	305
						June						
204	11.9	3.0	115	14	0.5	92	6.8	2.8	1.36	274	254	284
205[1]	-	-	-	-	-	-	-	--	-	-	-	-
206[2]	-	-	-	-	-	-	-	--	-	-	-	-
207	11.6	3.2	84	16	0.5	103	6.8	2.9	1.61	209	237	314
208	11.9	2.2	131	12	0.4	111	7.5	3.2	1.78	204	233	300
209	11.2	4.1	98	14	0.5	101	7.1	2.7	0.86	187	283	336

[1,2] Terminated at the end of May - open mare.

Table 21. Mean Values of Blood Constituents for Mares.

Month	Gr.	Ca (mg/dl)	P (mg/dl)	Gluc (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)	Chol (mg/dl)	TP (gm/dl)	Alb (gm/dl)	Bili (mg/dl)	Alk-P (mU/dl)	LDH (mU/dl)	SGOT (mU/dl)
Dec.	1	11.4	3.8	91	18	0.6	96	7.5	2.9	0.84	187	294	343
	2	11.0	3.9	89	17	0.6	89	7.4	2.9	0.89	196	294	330
Jan.	1	12.5	4.0	97	16	0.6	111	7.5	3.1	0.90	165	270	355
	2	12.4	4.0	93	15	0.6	112	7.5	3.1	0.95	164	272	344
Feb.	1	11.7	2.8	149	14	0.4	100	7.6	3.1	1.26	183	296	326
	2	11.8	3.1	192	14	0.5	102	7.4	3.1	1.59	178	276	305
March	1	11.7	3.5	103	14	0.8	108	7.3	3.0	1.03	176	256	328
	2	11.6	3.4	92	14	0.9	115	7.2	3.1	1.19	174	268	325
April	1	12.2	3.2	100	13	0.9	118	7.6	3.0	1.3	212	227	285
	2	12.5	3.4	101	13	0.8	123	7.5	3.3	1.6	203	248	295
May	1	11.8	3.4	88	13	0.6	117	7.0	2.8	1.33	311	343	315
	2	11.7	3.5	92	13	0.7	127	6.8	2.8	1.73	252	311	301
June	1	11.3	2.3	132	14	0.65	95	7.1	2.9	1.22	338	326	294
	2	11.6	3.1	107	14	0.5	102	7.0	2.9	1.40	218	252	308

Table 22. Blood Chemistry Profiles for Group 1 Foals

Foal#	Ca (mg/dl)	P (mg/dl)	Gluc (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)	Chol (mg/dl)	TP (gm/dl)	Alb (gm/dl)	Bili (mg/dl)	Alk-P (mU/dl)	LDH (mU/dl)	SGOT (mU/dl)
						Day 1						
F-69	11.9	4.9	143	10	0.9	143	5.4	3.3	3.68	1650	309	172
F-79	12.0	4.9	160	12	0.6	211	5.7	2.7	1.79	1600	400	145
F-89	11.9	5.5	130	8	1.1	158	5.8	3.0	3.58	2700	501	184
						Day 2						
F-69	11.4	6.0	176	5	0.7	162	5.1	3.3	3.89	1250	347	243
F-79	12.5	6.0	210	8	0.5	203	5.5	2.8	2.05	1500	387	195
F-89	11.6	5.9	171	6	0.7	170	5.6	3.0	3.90	2700	387	196
						Day 3						
F-69	11.3	6.3	173	4	0.5	171	5.1	3.3	3.60	1375	279	260
F-79	12.9	6.0	189	5	0.4	209	5.7	3.0	2.33	1550	390	241
F-89	12.4	6.5	149	6	0.7	181	5.6	2.9	3.69	3010	478	243
						Day 4						
F-69	11.7	6.8	179	4	0.8	197	5.3	3.4	3.44	1250	273	270
F-79	12.2	5.9	169	7	3.6	206	5.5	2.8	1.99	975	353	216
F-89	12.3	6.9	185	7	0.2	3.7	5.2	3.1	3.60	1031	365	224
						Day 30						
F-69	12.1	5.8	86	8	0.8	161	6.2	2.9	1.57	546	444	340
F-79	13.3	8.5	161	6	0.7	196	5.3	2.9	1.64	1030	495	308
F-89	11.4	5.6	163	6	0.6	151	5.6	2.8	1.42	610	372	270

Table 23. Blood Chemistry Profiles for Group 2 Foals

Foal #	Ca (mg/dl)	P (mg/dl)	Gluc (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)	Chol (mg/dl)	TP (gm/dl)	Alb (gm/dl)	Bili (mg/dl)	Alk-P (mU/dl)	LDH (mU/dl)	SGOT (mU/dl)
						Day 1						
F-204	12.3	5.8	103	12	1.0	263	4.8	3.1	3.75	750	503	125
F-207	12.2	4.8	95	13	0.9	186	5.8	3.1	3.66	975	346	262
F-208	12.8	5.2	191	13	1.1	218	5.1	3.1	3.99	3240	463	166
F-209	12.6	5.6	141	12	1.1	156	5.4	2.9	3.28	900	329	190
						Day 2						
F-204	10.9	6.2	157	9	0.4	324	5.9	3.0	5.00	1150	373	229
F-207	11.0	6.0	135	7	0.3	172	6.0	2.9	3.17	1414	283	254
F-208	11.4	6.6	165	13	0.9	249	5.8	3.0	2.75	2160	470	211
F-209	11.2	6.9	185	14	0.5	351	5.3	3.3	5.60	1125	446	201
						Day 3						
F-204	12.3	6.2	188	6	0.2	305	5.8	3.1	3.38	769	329	260
F-207	11.7	6.6	125	6	0.5	183	6.2	3.0	2.60	1286	368	310
F-208	12.0	6.9	179	12	0.5	219	5.5	2.9	2.12	1782	468	219
F-209	11.9	6.3	185	9	0.2	317	5.0	3.1	3.72	1050	323	204
						Day 4						
F-204	11.7	5.6	143	9	3.2	271	6.4	3.7	2.58	877	268	185
F-207	12.0	6.3	150	5	0.4	187	6.3	2.9	2.58	1286	367	350
F-208	11.9	7.1	148	9	1.0	257	6.0	3.1	3.07	1620	484	260
F-209	12.1	9.2	62	5	1.1	198	5.8	3.0	4.11	2176	571	258
						Day 30						
F-204	12.2	7.2	147	12	0.2	228	5.9	3.1	1.63	540	335	334
F-207	12.8	7.8	150	11	0.5	198	6.1	3.1	1.05	670	316	330
F-208	12.5	7.2	163	6	0.3	299	5.9	3.0	1.43	750	267	300
F-209	12.6	7.8	178	10	0.7	189	5.3	3.1	1.74	547	322	348

Table 24. Mean Values of Blood Constituents for Foals.

Day	Group	Ca (mg/dl)	P (mg/dl)	Gluc (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)	Chol (mg/dl)	TP (gm/dl)	Alb (gm/dl)	Bili (mg/dl)	Alk-P (mU/dl)	LDH (mU/dl)	SGOT (mU/dl)
1	1	11.9	5.1	144	10	0.9	171	5.6	3.0	3.02	1983	403	167
	2	12.5	5.4	133	13	1.0	206	5.3	3.1	3.67	1466	410	186
2	1	11.8	6.0	186	6	0.6	178	5.4	3.1	3.28	1817	374	211
	2	11.0	6.4	161	11	0.5	274	5.8	3.1	4.13	1462	393	224
3	1	12.2	6.3	170	5	0.5	136	5.5	3.1	3.21	1978	382	248
	2	12.0	6.5	169	8	0.3	256	5.6	3.0	2.96	1221	372	248
4	1	12.0	6.5	178	6	1.5	237	5.3	3.1	3.01	1085	230	237
	2	11.9	7.1	126	7	1.4	228	6.1	3.2	3.09	1490	423	263
30	1	12.3	6.6	138	7	0.7	169	5.7	2.9	1.54	729	437	306
	2	12.5	7.5	160	10	0.4	229	5.8	3.1	1.46	627	397	328

Table 25. Blood Chemistry Profile by Electrophoresis - Foals

Foal #	Gamma (gm/dl)	Beta (gm/dl)	Alpha-2 (gm/dl)	Alpha-1 (gm/dl)	Alb (gm/dl)	TP (gm/dl)
F-69	0.7	0.9	0.4	0.1	3.3	5.4
F-79	1.3	0.9	0.5	0.1	2.9	5.7
F-89	1.0	0.9	0.5	0.2	3.2	5.8
Mean	1.0	0.9	0.5	0.1	3.1	5.6
F-204	0.05	0.6	0.4	0.05	3.7	4.8
F-207	0.2	0.7	0.3	0.0	4.6	5.8
F-208	0.7	0.7	0.4	0.1	3.2	5.1
F-209	0.9	0.8	0.3	0.0	3.4	5.4
Mean	0.5	0.7	0.4	0.04	3.7	5.3
F-69	0.8	0.7	0.3	0.1	3.4	5.3
F-79	1.1	1.1	0.5	0.1	2.8	5.5
F-89	0.5	0.7	0.3	0.1	3.5	5.2
Mean	0.8	0.8	0.4	0.1	3.2	5.3
F-204	1.1	1.0	0.5	0.0	3.8	6.4
F-207	1.1	0.9	0.5	0.1	3.7	6.3
F-208	1.1	1.0	0.7	0.1	3.1	6.0
F-209	0.9	1.0	0.6	0.1	3.2	5.8
Mean	1.1	1.0	0.6	0.1	3.5	6.1

within the normal range. Group 2 foals showed significantly higher † ($P < 0.10$) BUN concentrations than Group 1. However, since neither group displayed any abnormalities in this regard, it is not evident that these results are indicative of any difference in renal function caused by nutritional status.

Fonnesbeck and Symons (1969) found BUN concentrations to be a reflection of protein content in the feed. This question of significant difference in dietary protein between groups of foals will be discussed in further detail when total plasma proteins are dealt with.

(e) Lactic Dehydrogenase (LDH)

There were no significant differences noted for groups of mares (Table 21) or foals (Table 24) in serum levels of the enzyme LDH. However, there did appear to be a higher level of LDH activity among foals during the first few days following parturition. Correlations intended to show any relationship of this nature were not high, -0.41 and -0.35 for groups 1 and 2 foals as shown in Appendix A, Figures 1 and 2 . This lack of strong correlation is appearently largely due to within group variation. Generally speaking, it would appear that an elevated LDH activity may be expected due to the foal's greater dependence on glycolysis for energy during the first few days of life. Naturally the degree of elevation would tend to depend on the adequacy of colostrum and milk from their respective dams; thus influencing the degree of dependence on glycolysis (Lehninger, 1970). With this in mind, it is my opinion that serum LDH concentrations are potentially good indicators of the relative performance of mares in terms of milk production. The fact that no significant differences in LDH activity were noted for the two groups of foals would appear to indicate that the performance of the mares, at least during the initial stages of lactation, was not significantly affected by the levels of feed imposed during the course of this trial.

(f) Alkaline Phosphatase (Alk-P)

There were no significant differences between groups of mares (Table 21) or foals (Table 24) in levels of serum Alk-P. Tests of significance were not done for mares as compared with foals. The overall mean value of 1386 mg/dl for foals was much higher than mares 196 mg/dl for Alk-P activity. Presumably, this is due to the greater osteoblastic activity in growing bone. Correlations of Alk-P activity versus time after parturition for foals, groups 1 and 2, were -0.48 and -0.58 respectively as shown in Appendix A, Figures 3 and 4. There did not appear to be any marked differences in Alk-P activity between pregnant and open mares.

(g) Serum Glutamic-Oxaloacetic Transaminase (SGOT)

The SGOT concentrations were not significantly different between groups of mares (Table 21) or foals

(Table 24). Comparing SGOT activity in foals to elapsed time after parturition revealed correlation for groups 1 and 2 of 0.66 and 0.76 respectively (Appendix A, Figures 5 and 6. Presumably this is due to the rapid development of tissue in the growing animal, necessitating increased activity of SGOT. Wolff et al (1969) points out that SGOT concentrations are occasionally used to indicate alterations of cell necrosis in many tissues. The reaction of SGOT to disease appears to be an increase in variability rather than a marked increase in activity.

Mares from both groups had serum levels generally within previously established normal ranges (Wolff et al, 1969 and Medway, 1969). In addition there did not appear to be any difference between pregnant and non-pregnant mares.

(h) Total Protein (TP)

The analysis of serum total protein reveals that the levels in group 1 mares were significantly ($P < 0.05$) higher than the group 2 mares (Tables 21). No significant differences were found in serum total protein levels of foals (Table 24). Graphical representation of changes in serum total protein are given in Appendix A, figures 7 and 8.

Interpretations of these results are made somewhat difficult by some confusion in the literature; especially with regard to what the serum total protein response should be to disease stress. Wolff et al,(1969) found that there was no difference in total protein between groups of diseased horses versus groups of healthy horses. Medway (1969) was unable to explain patterns in total protein, albumin and globulin concentrations among 60 horses.

Mean serum TP values of 7.28 g% (Medway, 1969) for mixed light horses and 7.46 g% (Wolff et al, 1969) for mares, suggests that both group 1 and 2 mares had serum TP levels within an acceptable range of the previously established norms.

Bowland (1974) points out that measurement of plasma proteins are of limited value in diagnosis of disease stress. Presumably, this is because the globulin fraction of serum proteins is small. The largest portion of total serum proteins is made up of albumin, (Lehninger, 1970). Fonnesbeck and Symons (1969) found that plasma protein concentrations did not significantly respond to dietary protein levels. However, in terms of our own experimental data, we are presented with very few alternative explanations, in spite of the findings of Fonnesbeck and Symons.

Considering there was virtually no change in albumin levels between groups, it may be assumed that the non-albumin fraction was responsible for the differences noted in total protein. One explanation would be that the higher serum total protein is a reflection of a higher nutritional plane via the presence of a higher concentration of metabolized amino acids in the blood. This is not to say that the group 2 mares were in a deficient state, in terms of protein. The decision as to whether or not total protein can be used to evaluate a diet's adequacy must be based on information derived from a wider range of nutritional levels. To rephrase, I feel that while total protein may have potential to be calibrated in such a way as to indicate relative protein status, it can only apply to the protein fraction of a ration and not to the other essential constituents of a diet.

(i) Bilirubin (Bili)

No significant differences were observed among the bilirubin levels in the serum of mares, (Table 21). Values for these mares ranged between approximately 0.5 - 3.0 mg per 100 ml. However, there was a trend for bilirubin levels to rise approaching parturition and fall rapidly after foaling, as shown in Appendix A, Figures 9 and 10. This is in agreement with the findings of Wolff et al,(1969)and Medway,(1969). Although these workers were not dealing with pregnant mares and therefore did not note the tendency for bilirubin to rise approaching parturition.

Blood serum samples from the foals also did not show any significant difference in bilirubin levels, Table 24. For the most part bilirubin levels were higher in the foals than in the mares and definitely tended to increase with time. At no time, for either group of mares and foals, did the serum bilirubin levels rise to concentrations indicated by Ford and Gopinath (1974) as being indicative of jaundice or liver malfunction.

Bilirubin is one of the major end products of hemoglobin decomposition. Bilirubin itself is relatively insoluble in the body fluids. As a result, it combines with some of the plasma proteins, mainly albumin and to a lesser extent alph-globulin, and is transported in this combination throughout the blood and interstitial fluid, (With, 1968).

It is generally assumed that all bile pigments formed in the organism are excreted with the bile. However, if experimental findings obtained with horses and cattle are considered, this assumption may not always hold true. With,(1968) refers to work by Bierthen (1906) that shows that the horse excretes only a minimum amount of bile pigments with bile, has a high serum bilirubin level and, under normal conditions, bile pigments are not excreted in the form of tetrapyruvole pigments but that further degradation to similar compounds occur. Working with horses, With (1968) noted that Cornelius et al (1960) found bilirubin levels to fluctuate as much as 25 mg per 100 ml of bile. He maintains that this wide variation is primarily due to the fact that the horse has no gall bladder and the technical difficulties associated with collections.

Garner (1953), as cited by With (1968), found that serum samples of normal cattle showed only traces of bile pigments. Further, he found that hemolysis will result in serum bilirubin concentrations of 7 - 8 mg per 100 ml, lesions of liver parenchyma or bile duct obstruction cause only insignificant bilirubinemia. This would appear to indicate that these animals have, to some degree, developed the ability to further degrade bilirubin, particularly the conjugated type. Whether or not the marked fluctuations found in serum bilirubin levels in horses is associated with this phenomenon has not yet been studied.

(j) Cholesterol (Chol)

Blood chemistry profile analyses reveals no significant differences in serum cholesterol levels of the two groups of mares (Table 21). Anderson et al (1952) points out that the primary determinant of serum cholesterol levels in the blood of man is caloric intake associated with weight loss or gain. This relationship was further elaborated on by Mann et al (1955) when it was shown that feeding healthy young men twice their normal caloric intake, failed to influence their serum cholesterol levels as long as they were allowed to increase their energy expenditure accordingly. From this information we may consider two alternate conclusions. Restriction of diet did not result in any discernable difference in caloric intake relative to requirements, thus implying that NRC guidelines for energy were higher than absolutely necessary under the environmental conditions of this experiment. The second alternative may have been that the mares in group 1 (high energy level) were more active than group 2 mares, thus reducing their serum cholestrol levels accordingly. From observation of the mares during the course of this experiment, no consistent behavioural differences were noted at any time.

Analyses of variance showed significant ($P < 0.05$) differences for serum cholesterol in foals (Table 24). Examination of the plotted data (Appendix A figures 11 and 12 shows a large difference at one day, decreasing slightly at two and three days after birth. Blood samples at 30 days showed that serum cholesterol levels which were initially lower in group 2 had increased to the point that both groups were almost identical. This would suggest that a significant difference in terms of caloric intake was experienced between groups of foals. However, the question of whether or not this difference is representative of an energy deficiency during the first few days post partum cannot be answered on the basis of serum cholesterol levels alone. Furthermore, whether this difference in caloric intake is due to increased milk production of group 1 mares or excessive protein intake of group 1 foals, cannot be answered directly.

There is evidence to suggest that increased dietary protein, in horses, can result in increased levels of serum cholesterol (Reitnour and Treece, 1971). Presumably, this increase in protein levels is treated as being excessive by the horse and is subsequently degraded to produce energy, ostensibly for fatty acid synthesis. If this, in fact, were the case for group 1 foals, regardless of the cause, then significant differences noted for serum cholesterol need not be indicative of failure to meet NAS-NRC (1973) energy requirement levels. However, it should be repeated that NAS-NRC (1973) may not necessarily be adequate in terms of performance, for example, in the showring, for all horses. Furthermore, any nutritional differences between groups of horses at this early stage of life may have long term consequences which should not be ignored merely because the extent of these differences are not known.

The significance of these serum cholesterol levels are confounded by the results obtained for serum BUN. The serum BUN levels were significantly higher in group 2 foals (Table 24). Presumably this is due to a higher dietary protein intake. This conflicts directly with the implications derived from the results for serum cholesterol.

Examination of blood serum constituents of foals by electrophoresis (Table 25) did not reveal any significant differences between groups of foals.

Results of the reproductive study, for the parameters measured, suggest that, while some differences were noted between groups of mares and foals, the lower nutritional regime (Group 2 mares) supplied nutrients of sufficient quality and quantity to maintain pregnant mares through the reproductive phases measured.

DIGESTIBILITY STUDIES

Digestibility studies consisting of three experimental trials, using total and grab sample collection techniques, and one rate of passage of digesta experiment were conducted on four geldings in each trial.

Rate of Passage

There has been a variety of materials used for rate of passage studies that were designed to reflect the rate of movement of digesta through the gastro-intestinal tract of the horse. There has been almost equal variety in estimates of rate of passage. It would appear that this variation is due to the types of indicators and feeds used. In an effort to use an indicator that would not diffuse through the GI tract at a rate different than the rate of passage of digesta, it was decided to use a material of similar bulk density that would also be easy to identify. The material that was selected for this experiment was cloth cut up into strips.

The results of these trials are summarized in Table 26. The mean rate of passage found was 71.1 h and 69.3 h for replicates 1 and 2 respectively. These estimates agree closely with Hintz and Loy (1966) who found elapsed time for the passage of styrofoam particles of 72 h. VanderNoot et al (1967) and Pulse et al (1973)

Table 26. Rate of Passage - Geldings

Horse	Elapsed Time (Hours)* Replicate I	Elapsed Time (Hours)* Replicate II
1	73.25	68.0
2	67.08	72.0
3	--	--
4	73.0	68.0
Mean	71.1	69.3

* Elapsed time refers to the duration time from when the cloth strips were fed until first cloth strip was detected in the feces.

found a retention time of 96 h using chromic oxide and polyethelene markers. Carbon granules used by Alexander (1946) appeared in feces after 47 h. These results tend to suggest that both selective retention and diffusion of markers is a problem. The fact that rate of passage is markedly decreased by the addition of fibre (Hintz and Loy, 1966) and (Haenlein et al, 1966) only serves to accentuate the need for an accurate method for determining the rate of passage of feedstuffs for horses.

Digestibility Trial 1

This trial was designed to exactly replicate the digestibility study done with the mares, with two exceptions. Firstly, geldings were used and secondly, total collections were done in addition to insoluble ash collections.

Results (Table 27 and 28) show that the average total collections gave a lower estimate of apparent digestibility for both nitrogen and energy than the insoluble ash technique. This is different from results obtained by McCarthy et al (1974) working with pigs and Voghtman et al, (1975) working with chickens; where they found estimates of apparent digestibility to be higher by the total collection method, however, these results are in general agreement with estimates of apparent digestibility for cattle, Van Keulen (unpublished data). One possible explanation for this was the fact that waste of a small portion of feed was unavoidable with the horses.

Table 27. Apparent Digestibilities for Geldings - Trial 1

		Digestible Nitrogen (%)										
Horse	Replicate	3 day Total	5 day Total	7 day Total	Mean	SD*	Random Grab	3 day HCL	5 day HCL	7 day HCL	Mean	SD*
1	1	67.7	69.7	69.9	69.1±	0.70	76.8	75.8	75.1	73.8	74.9	0.58
2	1	86.6	77.6	77.2	80.5±	3.06	85.3	83.9	83.5	83.7	83.7±	0.11
3	1	81.1	78.9	78.3	79.4±	0.85	90.0	84.4	86.7	87.6	86.2±	0.95
4	1	75.6	76.8	76.5	76.3±	0.36	86.3	83.0	81.9	82.2	82.4±	0.32
1	2	73.7	71.3	74.0	73.0±	0.85	75.2	78.0	76.7	75.0	76.6±	0.87
2	2	85.0	83.3	83.8	84.0±	0.50	86.1	87.2	85.1	86.7	86.3±	0.63
3	2	90.6	87.6	87.1	88.4±	1.09	92.2	89.5	93.0	91.8	91.4±	1.02
4	2	86.6	84.5	84.5	85.2±	0.70	85.9	87.1	86.1	85.9	86.4±	0.37

* Standard deviations between collections for each horse.

Table 28. Apparent Digestibilities for Geldings - Trial 1

		Digestible Energy (%)								
Collection Period		3 day	5 day	7 day	Mean SD*	Random	3 day	5 day	7 day	Mean SD*
Method of Determination		Total	Total	Total		Gram	HCL	HCL	HCL	
Horse	Replicate									
1	1	64.9	68.8	70.3	68.0± 1.61	77.5	76.3	76.9	76.7	76.9± 0.35
2	1	83.9	73.1	72.9	76.6± 3.63	78.9	80.7	80.2	80.7	80.5± 0.17
3	1	80.4	75.6	74.6	76.9± 1.79	84.5	83.8	84.6	85.4	84.6± 0.46
4	1	74.5	74.9	70.7	73.4± 1.34	80.9	82.3	80.4	80.3	81.0± 0.65
1	2	71.3	68.7	71.8	70.6± 0.96	75.8	77.4	76.2	74.5	76.0± 0.84
2	2	80.4	80.6	79.8	80.3± 0.24	79.9	83.3	82.7	83.4	83.1± 0.21
3	2	89.5	86.9	85.9	87.4± 1.07	90.2	88.2	92.6	91.0	90.6± 1.28
4	2	81.6	80.3	80.3	80.7± 0.43	81.6	82.2	82.4	82.0	82.2± 0.11

* Standard deviations between collections for each horse.

This error would be reflected in the total collection calculations resulting in a lower estimate of apparent digestibility. However, since insoluble ash is represented as a ratio of the ash in the feed versus the ash in the fecal sample, this error would not be reflected in the estimate of apparent digestibility. Add to this the fact that any errors encountered in either the weighing of the feed or weighing of the feces are going to be reflected in the estimates by total collection but not in the insoluble ash technique; we are faced with the possibility that the predominance of residual error lies in the total collection rather than the insoluble ash technique.

Analyses of variance showed no significant difference in digestibility for method of determination. However, there were significant differences ($P < 0.05$) between animals and between replicates for the insoluble ash technique. There was significant difference ($P < 0.10$) only between animals for the total collection method. The reason for the lack of significant difference between replicates for total collection was due to the fact that the standard deviation and thus the error term was larger than the error term calculated for the insoluble ash data. The wider variation encountered in the total collections tends to support the suggestion that considerable error can be encountered by this method. However, for this trial these differences do not appear to be critical to the comparison of the two methods of collection.

Correlations of the raw data for nitrogen and energy determinations (Tables 29 and 30) show a very strong relationship of total to insoluble ash methods. These encouraging results lead us to suggest that the insoluble ash technique for determining apparent digestibilities has the potential for accurate prediction, based on the assumption that the total collection estimates are accurate.

Digestibility Trial 2

Information regarding the efficiency of feed utilization by light horses is minimal, (Robinson and Slade, 1974). There are a number of reasons for this situation, the most important of which is that the collection of the data necessary for the determination of digestibilities is a time consuming task which, relative to the labor involved, yields little information. Ideally, the digestibility of a feed for a particular animal would involve about the same amount of labor as feed testing for total nutrients. With this in mind, the possibility of obtaining accurate estimates of digestibility by a random grab method of collection, implies some fascinating applications.

As a result of the potential benefits to be accrued from the proven application of the random grab method, trial 2 was designed to compare estimates of

Table 29. Correlations of Digestible Nitrogen for Insoluble Ash vs. Total Collection - Trial 1

Total Collection				Insoluble Ash		
3 day	5 day	7 day	Random Grab	3 day	5 day	7 day
-	0.92	0.91	0.79	0.93	0.90	0.90
	-	0.99	0.85	0.98	0.94	0.94
		-	0.77	0.96	0.91	0.90
			-	0.89	0.94	0.96
				-	0.95	0.97
					-	0.98
						-

Table 30. Correlations of Digestible Energy for Insoluble Ash vs. Total Collection - Trial 1

Total Collection				Insoluble Ash		
3 day	5 day	7 day	Random Grab	3 day	5 day	7 day
1	0.83	0.79	0.74	0.87	0.83	0.84
	1-	0.94	0.84	0.93	0.91	0.90
		1	0.76	0.81	0.86	0.82
	–		1-	0.92	0.97	0.96
				-	0.96	0.96
					-	0.98
						1-

apparent digestibility by the insoluble ash technique by collection for four consecutive days. Mean digestion coefficients of 92.1 for nitrogen and 88.7 for energy (Tables 31 and 32) compare favourably with estimates obtained in trial 3 using diets based on the same formulations.

Analyses of variance showed no significant differences between days. This would suggest that estimates obtained by random grab collections are sufficiently accurate to eliminate the need for further collections. Standard deviations for the insoluble ash collections (Tables 31 and 32) are much lower than those encountered in Trial 3 and suggest that some of the errors noted in this later trial have affected insoluble ash results as well as total collection estimates. However, standard deviations for Trial 3 included replicate effects which were not included in these standard deviation calculations. The implication of this is that, provided the accuracy of the insoluble ash technique is verified, it would be possible to determine the apparent digestibility of a feed by merely taking one feed and one fecal sample in the field.

The results found in this trial are similar to results obtained by Vogtmann et al (1975) in that the random grab method of collection appears to accurately reflect results obtained from longer term collections.

Digestibility Trial 3

This trial was similar to trial 1 and was intended

Table 31. Apparent Digestible Nitrogen (%) Estimated from Insoluble Ash - Trial 2

Horse	Replicate	Day 1	Day 2	Day 3	Day 4	Total[*]	Mean	SD
1	1	89.4	90.0	88.6	89.2	89.6	89.4±	0.23
2	1	92.5	92.2	91.8	93.0	92.6	92.6±	0.35
4	1	92.7	92.9	92.1	91.2	91.8	92.1±	0.31
5	1	93.0	92.6	91.8	92.6	92.5	92.5±	0.19
1	2	91.3	89.7	91.3	93.0	91.0	91.3±	0.53
2	2	93.4	93.1	94.1	93.9	93.6	93.6±	0.18
4	2	93.4	92.7	93.3	92.3	92.4	92.8±	0.23
5	2	93.1	92.3	92.3	93.1	92.5	92.7±	0.16

[*] The reference to "total" refers to a cumulative subsample taken from the previous four days.

Table 32. Apparent Digestible Energy (%) Estimated from Insoluble Ash - Trial 2

Horse	Replicate	Day 1	Day 2	Day 3	Day 4	Total*	Mean	SD
1	1	88.8	88.2	87.7	87.8	88.5	88.2	0.21
2	1	89.6	89.1	89.7	90.1	90.0	89.7	0.18
4	1	89.6	88.5	88.5	86.8	88.2	88.3	0.45
5	1	89.0	89.3	87.8	88.4	88.5	88.6	0.26
1	2	89.9	88.9	89.0	92.1	89.6	89.9	0.56
2	2	89.2	87.4	90.2	89.1	89.0	89.0	0.45
4	2	88.9	87.9	88.7	86.7	87.4	87.9	0.41
5	2	88.6	87.5	87.7	88.3	87.7	88.0	0.21

* The reference to "total" refers to a cumulative subsample taken from the previous four days.

to add further information to the total collection insoluble ash relationship. However, geldings in this trial were fed a slightly different diet (Table 10). Therefore direct comparison of raw data was not possible.

Analyses of variance revealed no significant difference between methods of determinations. Significant differences ($P < 0.05$) between animals and between replicates were found for the insoluble ash technique. No significant differences were found in the total collection method. The suggested reason for this result is the same as was found for trial 1. In this case the difference in standard deviations for the two methods was much greater in this trial. In fact, the wide variation encountered with the total collection (Table 33) constituted a critical source of error. Variations for the insoluble ash were also larger due to replicate effect but remained lower than total collection variation. This would suggest that for whatever the reason, the insoluble ash offered more consistent estimates of digestibility. Therefore it would appear that this technique is less subject to error than the total collection method.

A number of possible sources of error for the total collection method have been discussed under trial 1. The fact that these errors were larger in trial 3 than the other trials is difficult to explain. I feel that the

Table 33. Apparent Digestibilities for Geldings - Trial 3 - Digestible Nitrogen (%)

	Total Collection							Insoluble Ash						
	Replicate													
Horse	1	2	3	4	5	Mean	SD	1	2	3	4	5	Mean	SD
1	65.3	72.9	77.0	82.5	77.1	75.0±	2.86	77.9	76.1	74.1	72.0	76.7	75.4±	1.04
2	81.2	83.4	85.3	80.2	85.4	83.1±	1.05	85.3	82.9	82.3	81.0	82.5	82.8±	0.70
6	86.5	83.2	84.3	76.7	75.8	81.3±	2.13	81.7	79.1	78.3	74.8	77.6	78.3±	1.11
7	83.3	76.2	73.6	73.7	69.5	75.3±	2.28	78.4	75.4	73.4	70.8	76.6	74.9±	1.31
						Digestible Energy (%)								
1	60.5	69.8	69.9	81.2	72.3	70.7	3.30	74.8	73.2	66.1	70.1	71.8	71.2	1.49
2	67.6	75.2	77.3	72.5	80.7	74.7	2.20	74.5	74.4	72.5	73.7	76.9	74.4	0.72
6	80.3	77.9	76.8	70.7	70.5	75.2	1.98	73.2	72.5	67.9	68.3	72.7	70.9	1.15
7	80.8	71.7	64.8	69.4	69.3	71.2±	3.05	75.0	70.7	64.4	66.0	76.5	70.6±	2.78

efforts that were made to improve the accuracy of fecal collections in this later trial may have actually confounded the results. For example, in trial 1 collections were made from a concrete floor. This condition precipitated prompt fecal collections and thorough washing of the floor area. The fact that the floor area was kept clean meant that a small loss of fecal material was unavoidable. Otherwise total collections appeared to be accurate. In this later trial collections were made with the aid of metabolism crates. Wood chewing became a serious problem in this trial and wastage of feed was increased. In addition, some stealing of feed was also encountered and difficult to completely eliminate. Furthermore, feed wastage and stealing is not a direct source of error in the insoluble ash method. However, any significant amount of bulk (ie: wood) added to the feces or feed waste and stealing will be reflected in the total collection estimates.

Pairwise correlations of data (Table 34) show that the relationship between insoluble ash and total collection estimates is only a weak one. This tends to confuse the encouraging results found in trial 1.

The initial objective of these experiments was to test the reliability of the insoluble ash method as it related to the presumably most accurate, total collection. Based on the awareness of the inherent errors associated with the total collection technique

Table 34. Correlations of Insoluble Ash vs. Total Collection for Digestible Nitrogen and Energy - Trial 3

Replicate	Nitrogen	Energy
1	0.48	-0.38
2	0.84	0.21
3	0.93	0.85
4	0.35	0.35
5	0.66	0.44

it is questionable whether this provides an adequate standard by which to compare other methods of determining apparent digestibilities. From the results of these trials it is my opinion that the total collections, if carried out with extreme care, can give very accurate estimates, but that total collection is not sufficiently reliable in all cases to be considered a base for comparison.

The horse industry is in a position where a great deal must be learned in terms of breed, sex and age differences regarding the digestibility of feeds, before precise recommendations based on estimates of nutrient requirements can be issued. This, in turn, implies the necessity of determining the value of feeds in a consistent and reproducable manner. It is with this problem of obtaining consistent results that the major value of the insoluble ash method lies. The insoluble ash technique displays considerable potential for use with large numbers of animals, due to the fact that the random grab sampling method eliminates the need for lengthy fecal collection periods. Clearly, this technique deserves further examination into its potential to accurately estimate apparent digestibility of feedstuffs for horses.

GENERAL DISCUSSION AND SUMMARY

An experiment was conducted at the Horse Husbandry Unit, Olds, Alberta. An additional experiment was completed at the Edmonton, Research Station, the University of Alberta, Edmonton, Alberta. The main objective of the first experiment was to assess the relative adequacy of a diet designed to meet NAS-NRC (1973) nutrient requirement guidelines throughout three phases of reproduction. The second experiment was designed to evaluate the reliability of the random grab insoluble ash technique for determining apparent digestibilities in horses.

The first experiment involved feeding a simple diet at two levels to two groups of mares. The first group of mares (group 1) received a ration at levels designed to meet NAS-NRC (1973) requirements for three phases of reproduction; early gestation, late gestation and early lactation. The second group of mares (group 2) received a ration, 15% less by weight than the first group.

In the second experiment, three separate trials were completed. The first trial (Trial 1) involved four geldings. They received an identical ration to that received by the mares in the first experiment. Total fecal collections were made over a period of seven days.

The comparison of feed intake and feces output provided for the calculation of apparent digestible nitrogen (DN) and energy (DE). Similar determinations for DN and DE were calculated by the insoluble ash method. Samples for insoluble ash analysis were taken as sub-samples of total collections. An additional fecal sample for insoluble ash was taken by a random grab selection. The two methods of determination were then compared in terms of repeatability of results and accuracy of prediction, using the total collection method as a control. The second trial (Trial 2) involved the comparison of digestibilities to period of collection using only the insoluble ash technique for analyses. The third trial (Trial 3) involved comparisons similar to Trial 1 except that the period of collection was for four days only. Rate of passage of digesta was estimated using cotton strips as markers.

The reproduction study with mares showed that some significant differences due to treatment effect were encountered; notably, serum total protein, urea nitrogen, cholesterol and milk protein samples. However, the majority of parameters measured did not indicate any trends in favour of the group 1 mares or foals. Furthermore, the significant differences that were encountered tended to conflict. As a result it would appear that for the periods measured, NAS-NRC (1973) Nutrient Requirements of Horses are in excess of minimum maintenance requirements for pregnant mares. However, the nutrients required by suckling foals to attain a maximum rate of gain were not dealt with.

The digestibility study with geldings involving three digestibility and one rate of passage trial provided evidence to suggest that the random grab insoluble ash technique can be used for the determination of apparent digestibilities in horses. In addition the rate of passage trial showed that for pelleted feeds the expected rate of passage of digesta would be approximately 72 hours.

Trial 1 showed that there was no significant difference between methods of determination of apparent digestibility nor were there significant differences noted for periods of collection for horses. Correlations of total collection and insoluble ash at three days were 0.93 for digestible nitrogen and 0.87 for digestible energy. Correlations for the seven day total collection and the random grab insoluble ash were 0.77 and 0.76 for digestible nitrogen and energy respectively.

Trial 2 showed no significant differences of apparent digestibilities using the insoluble ash technique for collection periods ranging from random grab to four days.

Trial 3 showed no significant difference between methods of collection. However, correlations for total collection compared with insoluble ash for digestible nitrogen and energy were generally less consistent than Trial 1 or the digestibility trial with mares. These correlations also showed much greater variation between replicates than any of the previous studies. The standard deviations for this trial were also larger than any other trial. The major source of variation in this study was from the total collection estimates of digestibility.

In general, these digestibility studies have shown that the insoluble ash technique provides more consistent estimates of apparent digestibility than total fecal collections. The random grab sampling method of insoluble ash determinations has shown the potential to consistently predict apparent digestibility similar to total collections and are in fact more reliable than total collections under conditions not well suited for total fecal collection.

The practical application of this particular finding is that a potential exists for estimating apparent digestibilities of large numbers of animals without prolonged confinement of the individual.

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APPENDIX A

LIST OF FIGURES

Figure 1. Effect of Age on Serum Lactic Dehydrogenase Concentrations for Group 1 Foals..

Figure 2. Effect of Age on Serum Lactic Dehydrogenase Concentrations for Group 2 Foals..

Figure 3. Effect of Age on Serum Alkaline Phosphotase Concentrations for Group 1 Foals..

Figure 4. Effect of Age on Serum Alkaline Phosphotase Concentrations for Group 2 Foals..

Figure 5. Effect of Age on Serum Glutamic - Oxaloacetic Transaminase Concentrations for Group 1 Foals........................

Figure 6. Effect of Age on Serum Glutamic - Oxaloacetic Transaminase Concentrations for Group 2 Foals........................

Figure 7. Effect of Reproductive Phase on Serum Total Protein Concentrations for Group 1 Mares..

Figure 8. Effect of Reproductive Phase on Serum Total Protein Concentrations for Group 2 Mares..

Figure 9. Effect of Reproductive Phase on Serum Bilirubin Concentrations for Group 1 Mares..

Figure 10. Effect of Reproductive Phase on Serum Bilirubin Concentrations for Group 2 Mares..

Figure 11. Effect of Age on Serum Cholesterol Concentrations for Group 1 Foals.........

Figure 12. Effect of Age on Serum Cholesterol Concentrations for Group 2 Foals.........

Figure 1. Effect of Age on Serum Lactic Dehydrogenase Concentrations for Group 1 Foals.

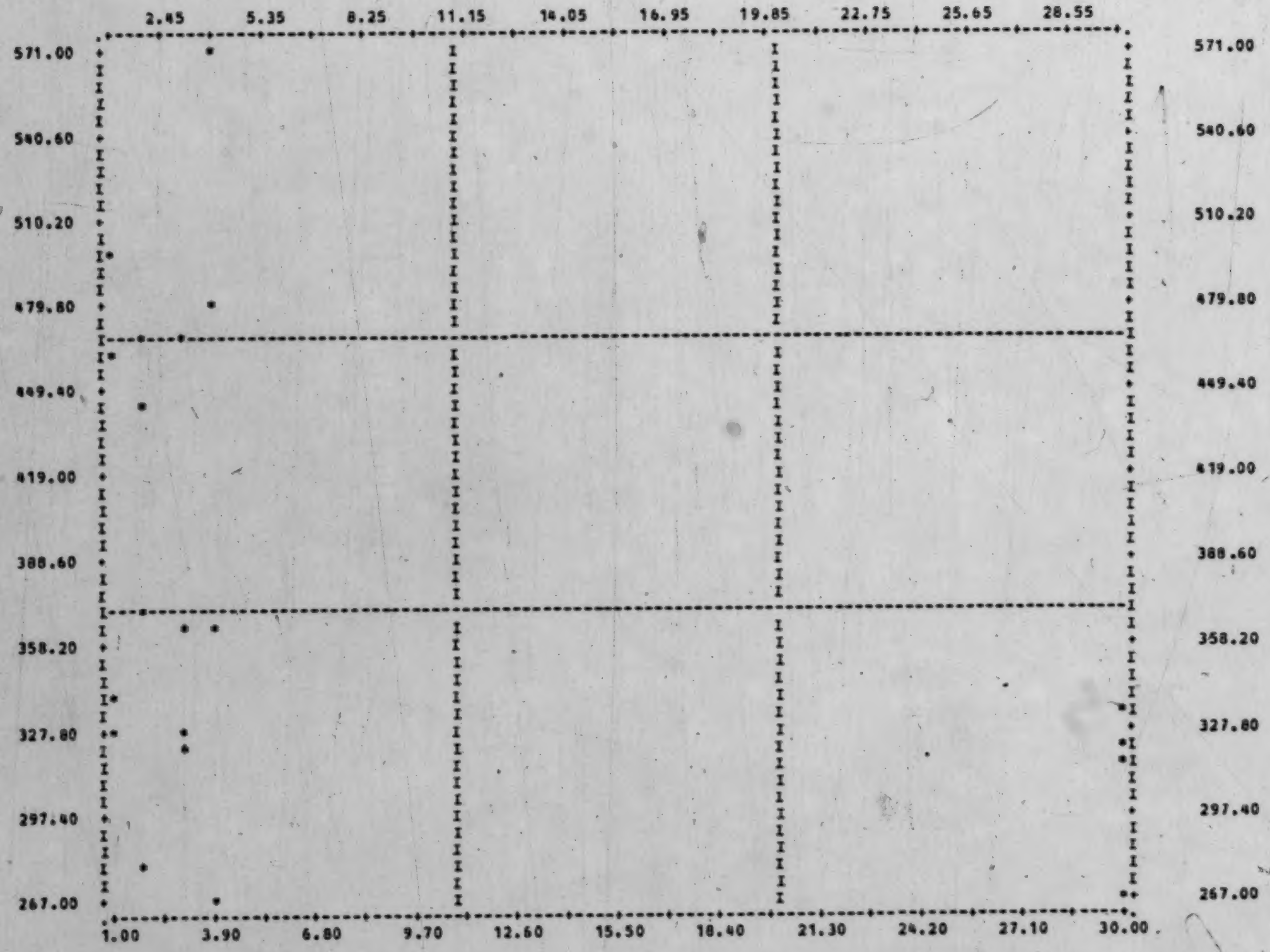


Figure 2.
Effect of Age on Serum Lactic Dehydrogenase Concentrations for Group 2 Foals

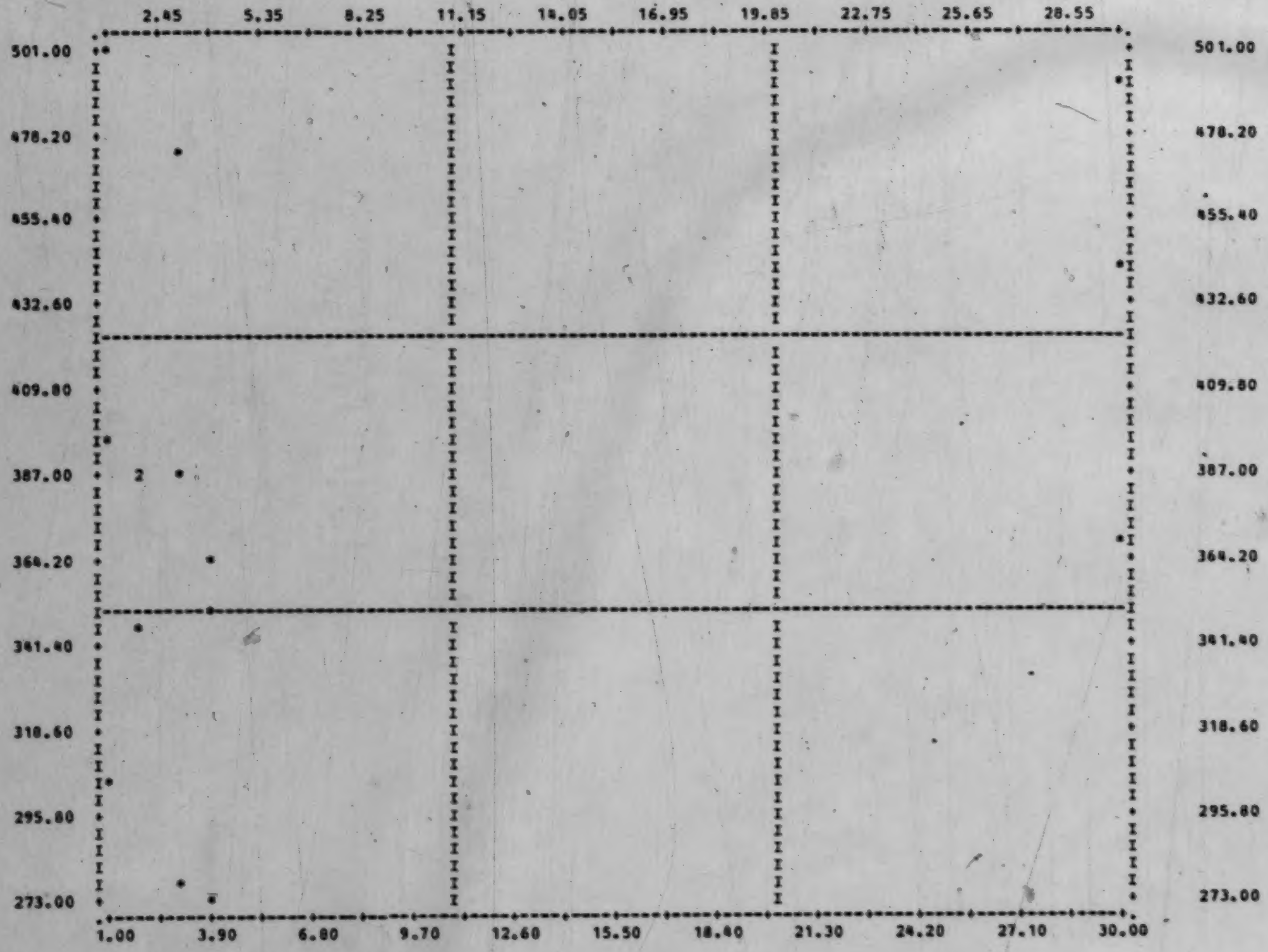


Figure 3. Effect of Age on Serum Alkaline Phosphotase Concentrations for Group 1 Foals.

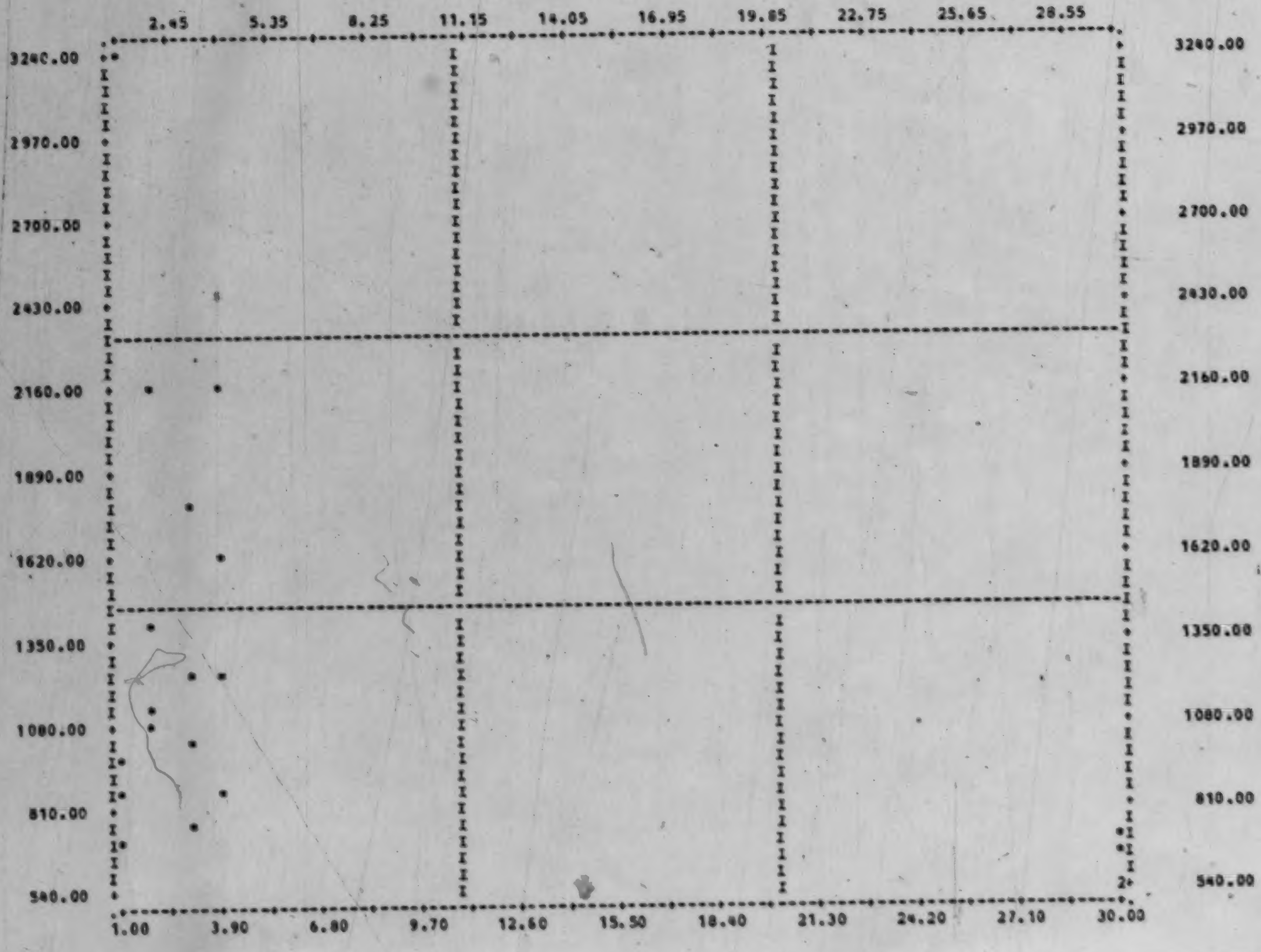


Figure 4. Effect of Age on Serum Alkaline Phosphotase Concentrations for Group 2 Foals.

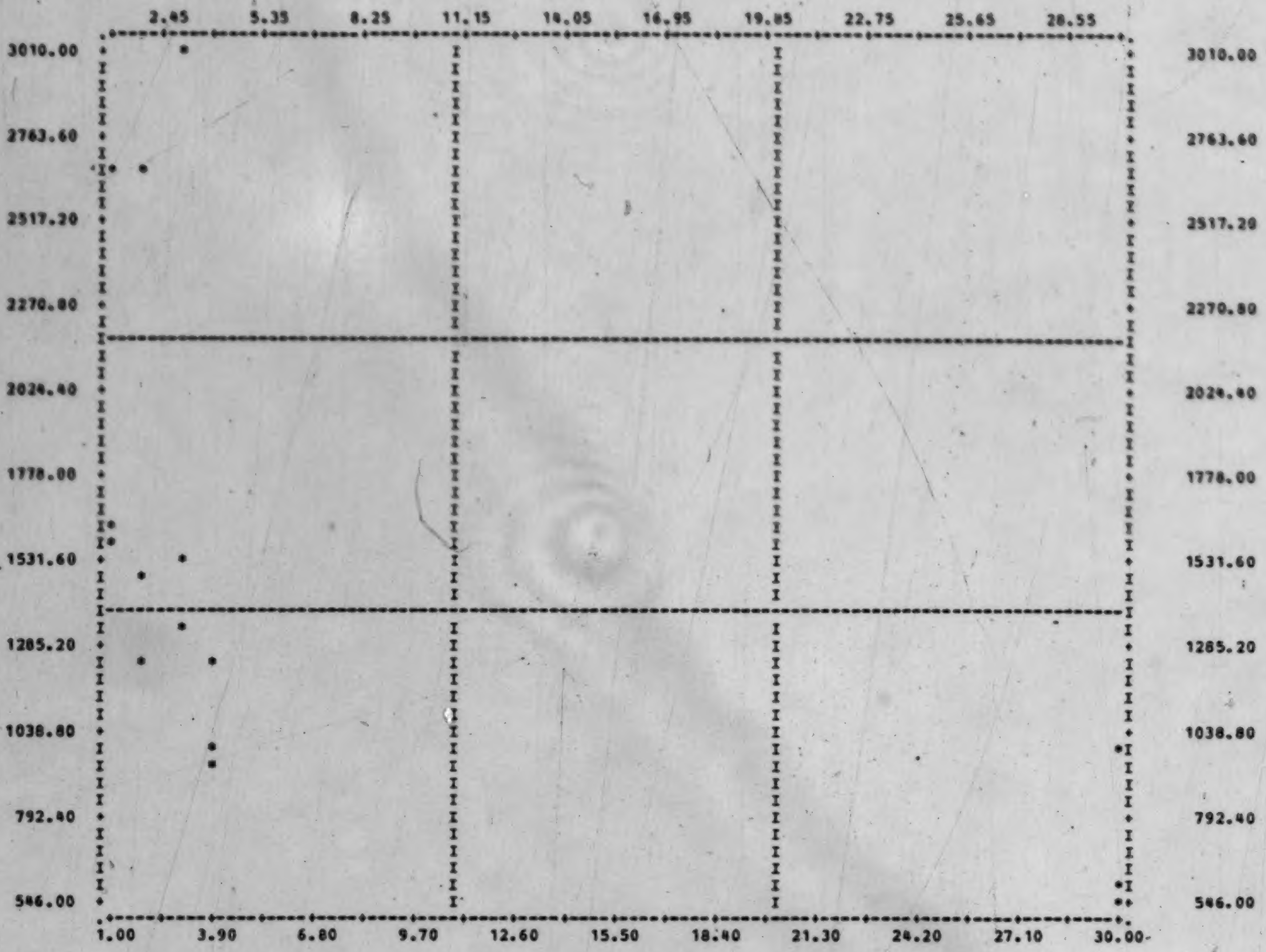
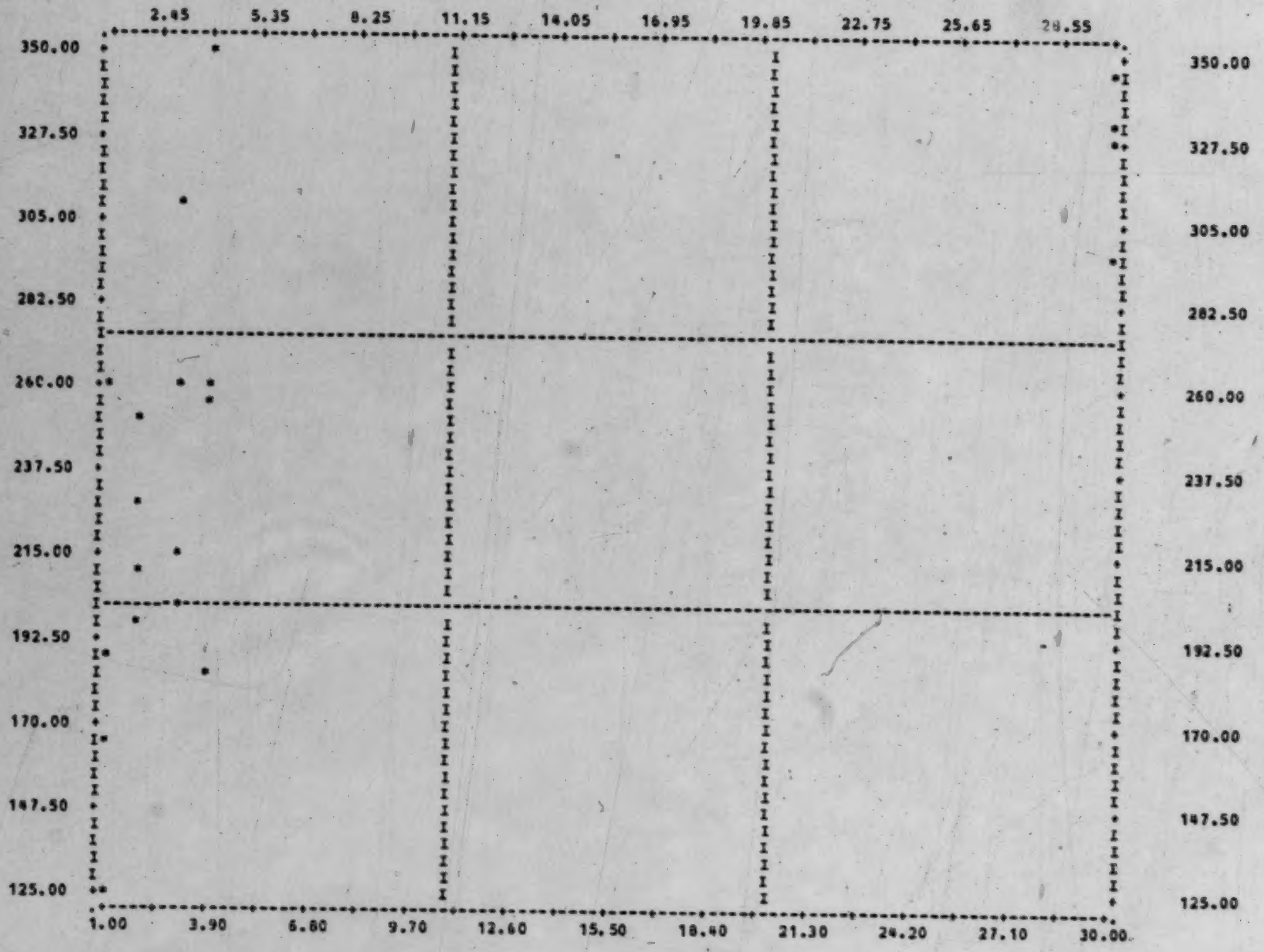


Figure 5. Effect of Age on Serum Glutamic-Oxaloacetic Transaminase Concentrations for Group 1 Foals.



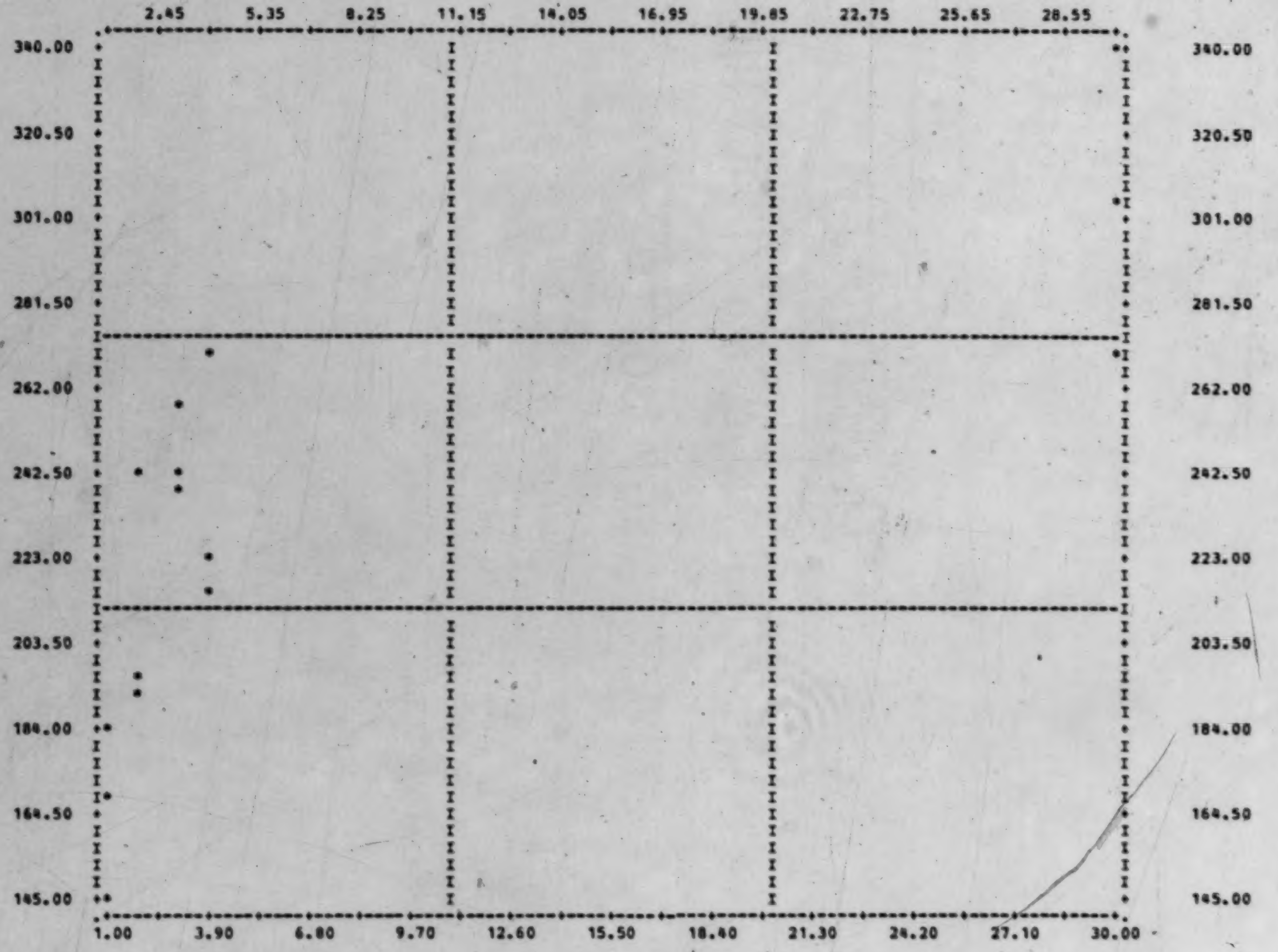


Figure 6. Effect of Age on Serum Glutamic-Oxaloacetic-Transaminase Concentrations for Group 2 Foals.

Figure 7. Effect of Reproductive Phase on Serum Total Protein Concentrations for Group 1 Mares.

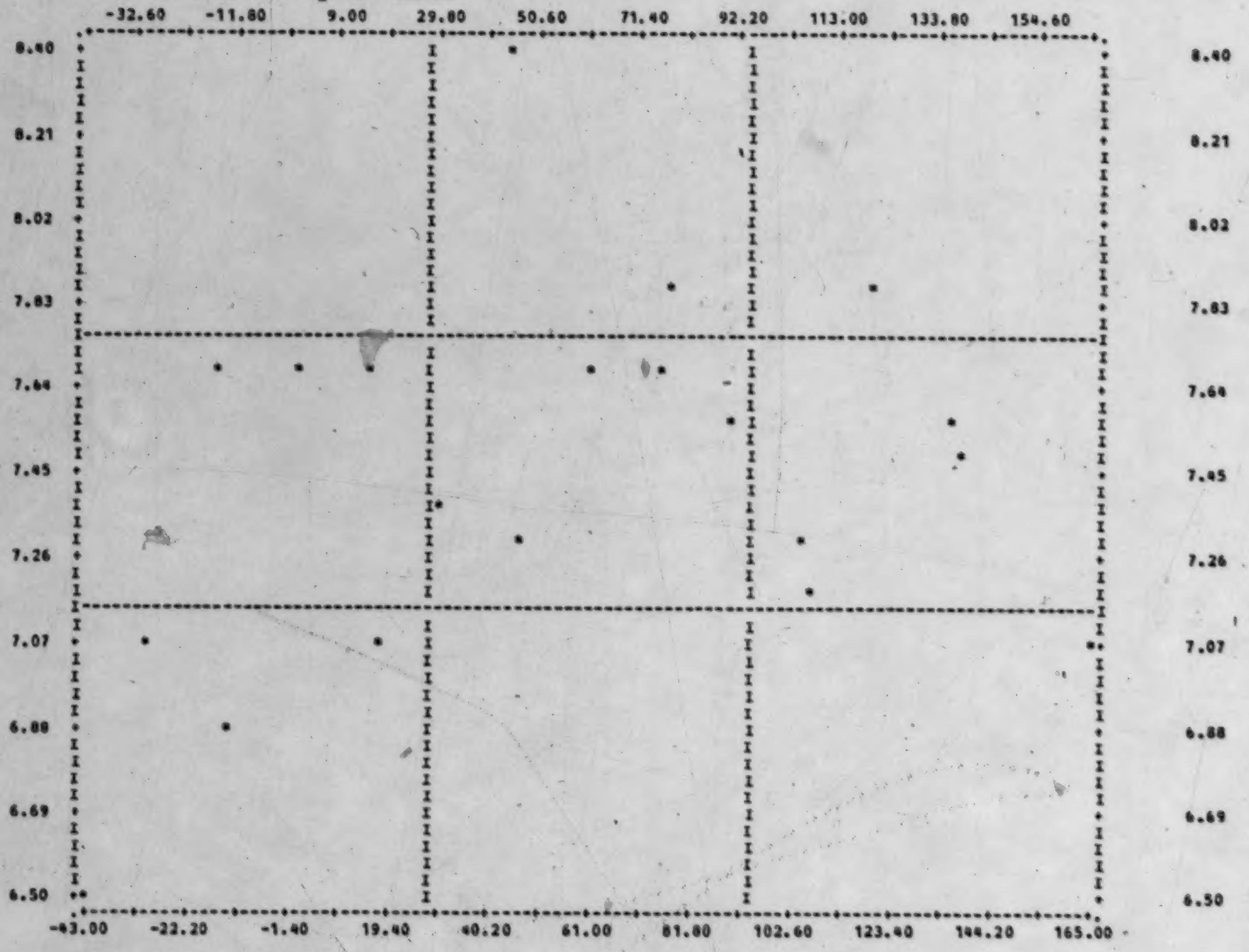


Figure 8. Effect of Reproductive Phase on Serum Total Protein Concentrations for Group 2 Mares.

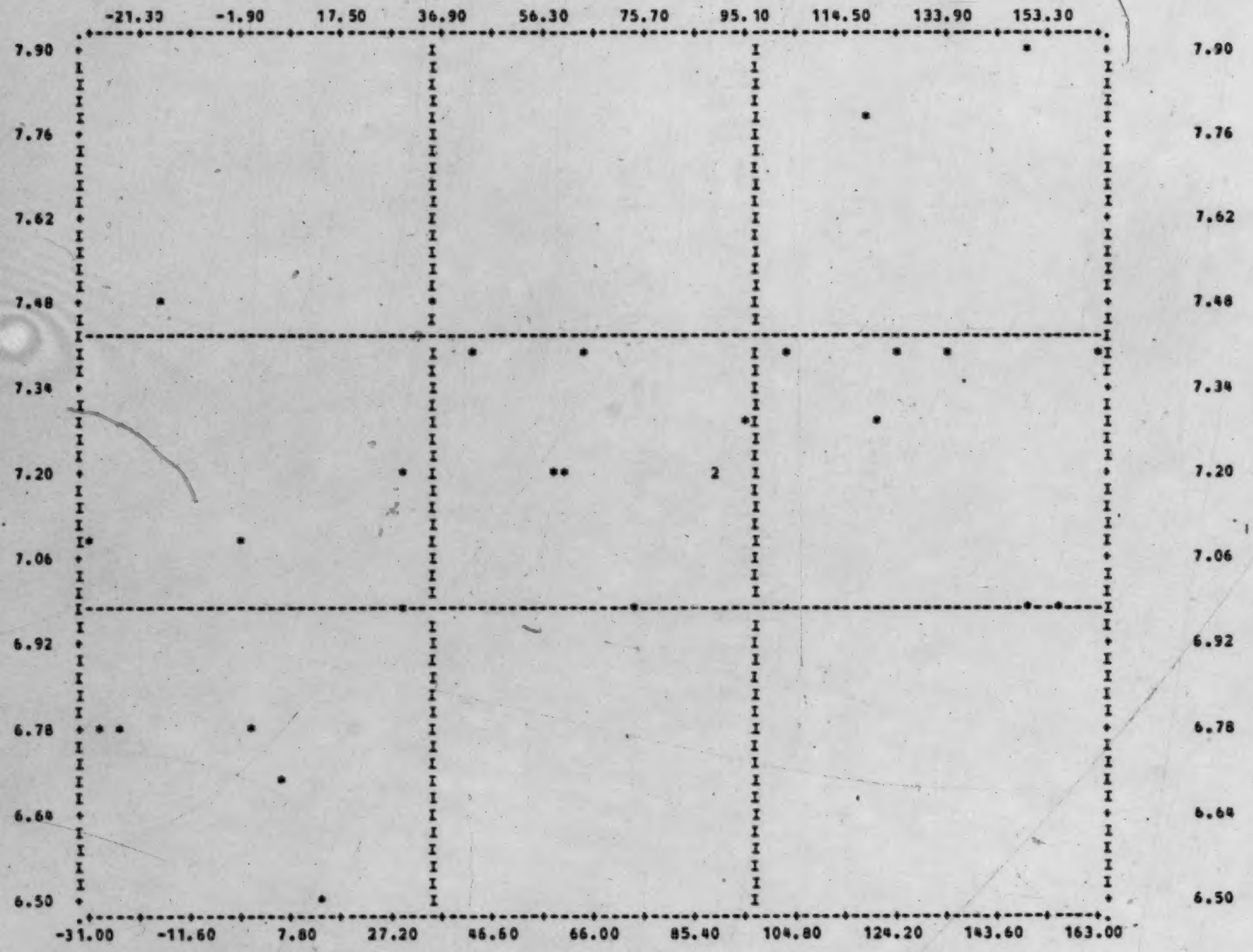


Figure 9. Effect of Reproductive Phase on Serum Bilirubin Concentrations for Group 1 Mares.

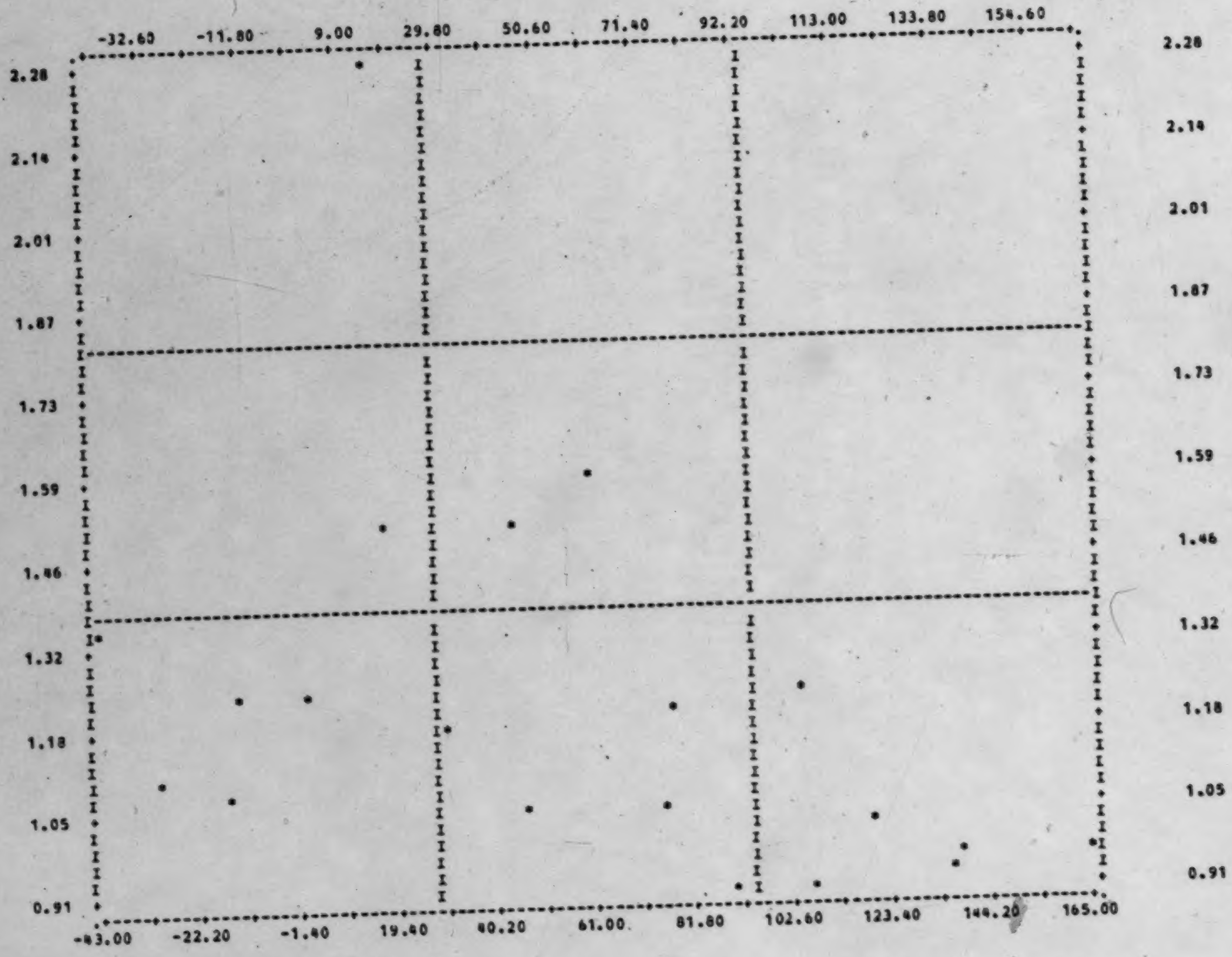


Figure 10. Effect of Reproductive Phase on Serum Bilirubin Concentrations for Group 2 Mares.

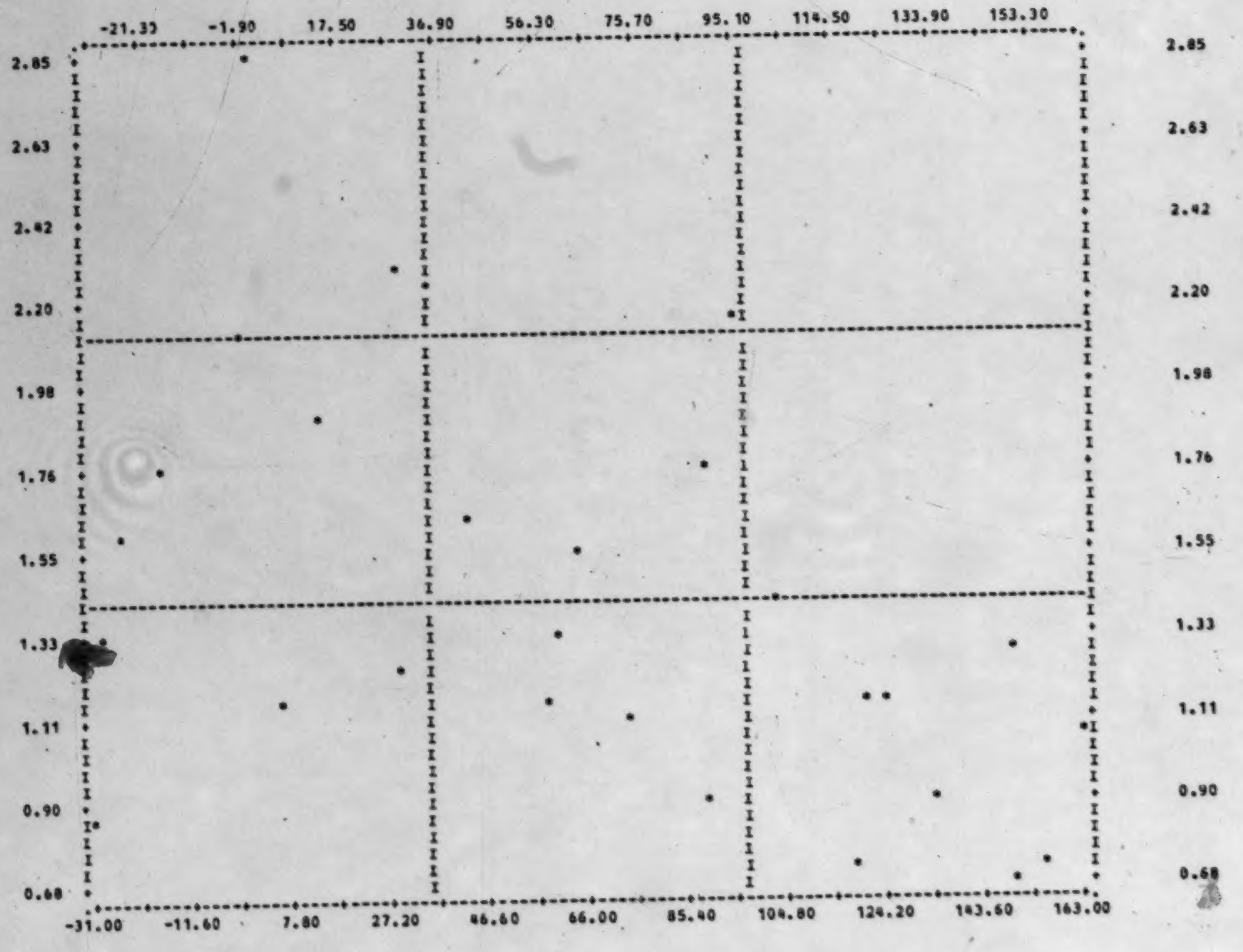


Figure 11. Effect of Age on Serum Cholesterol Concentrations for Group 1 Foals.

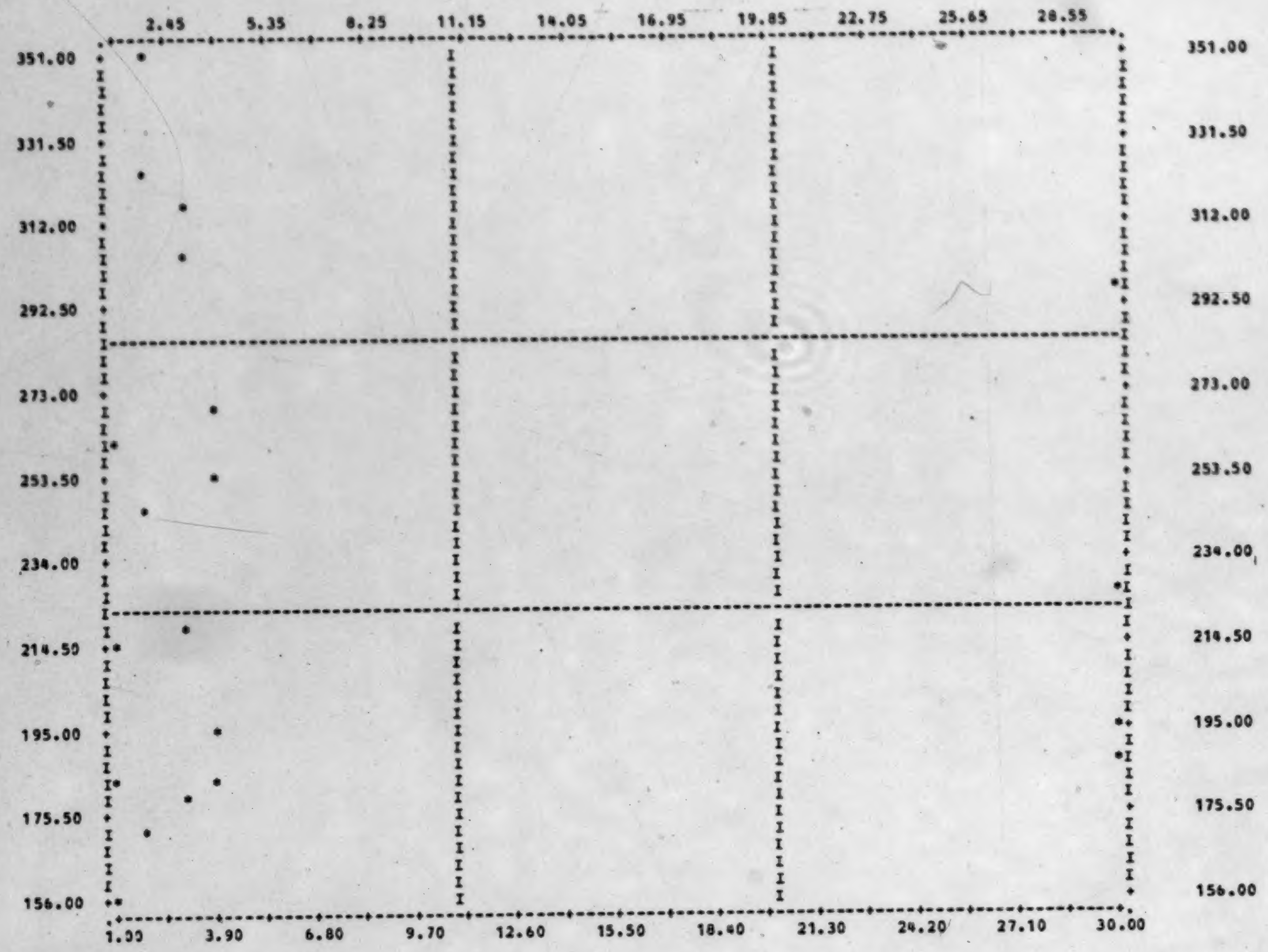


Figure 12. Effect of Age on Serum Cholesterol Concentrations for Group 2 Foals.